Interleukin 6 and 8 gene expression responses to resistance exercise and the correlation to muscle mass

Vivien Massie

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Interleukin 6 and 8 gene expression responses to resistance exercise and the correlation to muscle mass

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This thesis is presented in partial fulfilment of the requirements for the award of Bachelor of Science (Exercise and Sport Science) with Honours at Edith Cowan University, Western Australia.

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ABSTRACT

The post exercise inflammatory response is a key signalling mechanism regulating muscle protein synthesis. The purpose of this research was firstly to determine whether muscle mass in non-strength trained individuals was associated with the inflammatory muscle gene response after a single bout of eccentric muscle loading. Secondly, to determine whether changes in muscle cross-sectional area after a chronic increase in muscle loading (resistance training) is related to the inflammatory gene response to a single bout of muscle loading.

Eleven male participants (21.6 ± 4.1 years) volunteered for this study. Each participant completed a preliminary testing session that consisted of two triggered muscle biopsies, one immediately before and a second, one hour after a leg extensor resistance exercise bout, including the exercises leg press and leg extension. Eight weeks comprising 3 sets of 10 to 12 reps per set of training followed, where by all participants completed training at moderate intensity (MR; 70% 1 RM) or high intensity (HR; 90% 1 RM). The relationship between CSA, measured using CT scans of the subjects and gene expression of IL-6 and IL-8 were correlated. It was found that the change in IL-6 gene expression and pre-training CSA were related ($r=.61$, $p$ (one tailed) $< 0.05$), as were change in IL-8 expression and pre-training CSA were correlated ($r=.85$, $p$ (one tailed) $< 0.05$). For the MR group post-exercise IL-6 and IL-8 expression were correlated with pre-training CSA ($r=.93$, $p$ (one tailed) $< 0.05$ and $r=.90$, $p$ (one tailed) $< 0.05$, respectively).

For the HR group neither the change in IL-6 nor IL-8 expression correlated with pre-training CSA or any change in CSA. Individuals with the largest initial muscle mass prior to resistance training had the largest inflammatory response to training. However, there was no relationship between the change in CSA and change in gene expression.

Only those in the MR group with the largest initial muscle mass showed a large inflammatory response to the exercise. However they did not increase muscle mass the most after the training period.

The present research shows that those with a larger muscle mass have a greater inflammatory response to an exercise bout. However, those that have large increases in gene expression over the course of a single exercise bout do not necessarily show a greater increase in muscle mass over the course of a period of strength training.
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CHAPTER ONE

Introduction
1.1. Background

Humans with a greater muscle mass have been shown to both live longer and have a better functional capacity (Mazzeo et al., 1998). One reason for this may be that a greater muscle mass would result in an increase in metabolic rate (i.e. more metabolically active tissue) (Speakman & Selman, 2003), that leads to a decrease in the incidence of metabolic disease (Tarantino, Massa, Franco, & Pasanisi, 2008). Muscle has an endo-, para- and autocrine function that gives it the ability to produce proteins and hormones required by the muscle, and are also essential for the function of the whole body (Febbraio & Pedersen, 2005). Bone mineral density (BMD) can be greater in individuals who have a larger muscle mass (Mazzeo et al., 1998). In fact, BMD is more highly correlated with muscle mass than it is with peak strength (Gerdhem, Ringsberg, Akesson, & Obrant, 2003). Collectively these findings suggest that individuals who have a higher muscle mass are likely to live longer and have a better quality of life. Therefore, understanding the factors that influence muscle mass in humans may lead to the development of interventions that could influence longevity and quality of life.

There are two factors that influence muscle mass; muscle loading (Grund et al., 2001; Lee et al., 2008; Stenholm et al., 2008) and disease (Lee et al., 2008). Muscle mass will generally increase as an individual undertakes more physical activity (Tipton, Ferrando, Williams, & Wolfe, 1996), especially resistance training (Baar & Esser, 1999; Dreyer et al., 2006; Kubica, Bolster, Farrell, Kimball, & Jefferson, 2005). However, there was no correlation between ‘physical activity levels’ within the range of bedridden to normal working individuals and muscle mass in humans (Gerdhem, Ringsberg, Akesson, & Obrant, 2003). Therefore, factors other than normal physical activity must be important. There are two main hypotheses proposed to explain this finding;

1) different types of activity (e.g. resistance training) influences muscle mass in varying ways (Baar & Esser, 1999; Holm et al., 2008; Terzis et al., 2008), and/or
2) individuals can have different responses to the same activity patterns (Baar & Esser, 1999; Dreyer et al., 2006; Kubica et al., 2005).

With respect to hypothesis 2, muscle protein synthesis in response to a given bout of muscle loading varies between individuals (Baar & Esser, 1999; Dreyer et al., 2006; Kubica et al., 2005). This may lead to some individuals carry greater muscle mass and thus have a better health status. This hypothesis could be tested if a group of individuals with varied muscle mass, but similar activity histories and dietary intakes were tested to determine whether their protein synthesis response to a prescribed bout of muscle work were different. Recent advances in the understanding of the cellular processes involved in muscle protein synthesis have allowed the identification of several key signalling molecules. These signalling molecules can be arranged in a cascade where inflammation-inducing molecules, such as IL-6 and IL-8 are thought to be a prerequisite (further up the cascade) for protein synthesis, because inflammatory gene expression is linked to exercise-induced protein synthesis (Buford, Cooke, Shelmadine, et al., 2009; Buford, Cooke, & Willoughby, 2009; Nieman et al., 2001; Suzuki et al., 2003).

It has been shown that inflammatory gene expression is upregulated after resistance training (RT) (Buford, Cooke, & Willoughby, 2009; Izquierdo et al., 2009; Steensberg et al., 2000). Interleukin 6 (IL-6) and interleukin 8 (IL-8) are two such inflammatory genes that are thought to be required for protein synthesis. IL-6 and IL-8 have been seen to increase a few hours after RT (Akerstrom et al., 2005; Buford, Cooke, & Willoughby, 2009; Izquierdo et al., 2009), whereas other signalling molecules such as mTOR can have a peak upregulation 6 hours after RT (Parkington, LeBrasseur, Siebert, & Fielding, 2004). After prolonged exercise or eccentric-only exercise there can be a decrease in muscle mass so there may be an optimum window of inflammatory gene expression (Miles, Pearson, Andring, Kidd, & Volpe, 2007; Ostrowski, Schjerling, & Pedersen, 2000). This may support the theory that the inflammatory response, within a given range of gene expression has a rapid stimulus for protein synthesis, and therefore influences muscle mass, after given types of exercise.
1.2. Significance of the study

A greater muscle mass is associated with functional capacity and improved morbidity. In order to increase or maintain muscle mass in the general population it is important to determine the factors governing muscle mass. One theory is that each individual is predisposed to a given muscle mass because they have a unique response to a bout of physical activity, i.e. those with a greater muscle mass have a more optimum response of the intra-cellular protein signalling cascade. A minimum response is required, but a substantial response may be detrimental to this. Despite this, little research has investigated this relationship between muscle mass and intra-cellular protein signalling. This research is significant because it is the first to investigate the inflammatory response and it's relationship to muscle mass (cross-sectional area; CSA), and so by investigating this relationship further research can then be undertaken to discover how a training program can best influence an individual's muscle mass. This can be beneficial for a range of populations, including the elderly, in an attempt to decrease the risk of falls, and in slowing the rate of muscle wasting after bed rest.

1.3. Purpose of the Research

The purpose of the present study is firstly to determine whether individuals with greater muscle CSA show a unique inflammatory gene response to a bout of physical activity. And secondly to determine whether individuals with a greater inflammatory gene response to an intense exercise bout (resistance training; RT) increase their muscle CSA more than those with a lesser response after 8-weeks of RT.
1.4. Research Questions

1) Is there a relationship between the acute resistance training induced IL-6 and IL-8 gene expression and quadriceps muscle cross-sectional area in non-strength trained individuals?

2) After 8 weeks of high load lower extremity training (RT), is there a relationship between the change in muscle CSA and acute exercise-induced IL-6 and IL-8 gene expression?

1.5. Hypotheses

1) Participants with a larger muscle mass prior to training will have a greater inflammatory response (greater upregulation of IL-6 and IL-8 gene expression) to an exercise bout, than those with a smaller muscle mass.

2) Participants with a larger muscle mass prior to training will show a greater increase in quadriceps muscle cross-sectional area after 8 weeks of heavy RT.

1.6. Delimitations

The investigator imposed the participant delimitations of excluding participants that have had recent lower leg injury, also inclusion of males only. Only young and healthy participants were accepted. Therefore, other groups or the general population cannot directly apply the results from the current study. The race of participants was not taken in to consideration due to the nature of the present study as a pilot study to a longer training study. By discounting race as a factor this allowed a more over all evaluation of the effect of resistance training on gene expression and muscle size.
CHAPTER TWO

Review of Literature
2.1 Introduction

Maintenance of muscle mass is necessary for the performance of activities of daily living and most athletic tasks, and can influence quality of living. Clinically, increasing muscle mass can help in the recovery of patients from long-term bed rest (Lee et al., 2008), and in the elderly it is important in order to prevent falls, which is a primary risk factor for premature death (Lilley, Arie, & Chilvers, 1995). Gene expression may be a substantial determinant of muscle mass. Particularly, the early inflammatory response to some types of exercise, including resistance training has been shown to possibly be a key player in the initiation of protein synthesis (Izquierdo et al., 2009). Muscle mass is also important in the healthy population as it can improve athletic performance and improve general quality of life. The purpose of this review is to describe briefly the changes in gene expression associated with protein synthesis in skeletal muscle and serum; and to detail the importance of the expression of key genes related to the inflammatory cascade that regulate muscle size, as well as the strong influence of physical exercise.

2.1.1 Protein Synthesis and Protein Degradation Regulation

Protein synthesis is the process of accumulation of muscle and naturally occurs throughout the body, as it is required. Protein degradation, on the other hand is the process that works to break down the muscle. Both act in unison to balance the effects of the other to create a state of homeostasis.

Skeletal muscle consists of a complex network of signalling molecules and processes that influence protein synthesis and degradation within the muscle. There is a constant turnover of proteins in muscle, where a delicate balance of protein synthesis and protein degradation dictates the tendency for muscle acquisition or loss. With decreases in the work (i.e. a force produced over a muscle shortening distance) performed by a muscle, the rate of muscle mass accumulation decreases, affecting the balance between muscle synthesis and muscle degradation (Boonyarom & Inui, 2006). So as a muscle is not
stressed, less muscle is built and more is broken down (Christensen et al., 2008). The opposite occurs as the muscle does more work.

2.2 Muscle Cells

2.2.1 Cellular Processes

A muscle cell consists of numerous organelles that serve important functions within the cell. The nucleus and cytoplasm are both important for the synthesis of proteins. The process of transcription occurs in the nucleus and translation within the cytoplasm. Muscle protein synthesis begins with the copying of the gene needed from the deoxyribonucleic acid (DNA) (transcription), into messenger ribonucleic acid (mRNA). The mRNA then delivers the copy to the ribosome. Two sub-units join to form the ribosome, the platform on which translation occurs. Translation is the process whereby the transfer RNA (tRNA) and amino acids work, with the mRNA to form a protein. The following sections provide an overview of this process and a pictorial form in Figure 2.1.

2.2.2 The Nucleus

Muscle cells are multinucleated. Spread along the length of the cell (fibre) is many nuclei. Within a single nucleus, there are 23 chromosomes containing DNA. DNA consists of a long string of genes connected end to end. Each of these genes is the 'blueprint' for a specific protein. A signalling molecule is sent to a gene when needed, initiating transcription, which initiates the protein synthesis. After gene transcription, the mRNA translocates out of the nucleus to the ribosomes, which help to build the protein.
Figure 2.1. A simplified pictorial of transcription and translation within a muscle cell. A) the deoxyribonucleic acid (DNA) is transcribed into messenger ribonucleic acid (mRNA), acting as the master plan for the building of a protein. B) the transfer RNA (tRNA) attaches to a free amino acid (the building blocking in the process), in preparation for the building of the protein. C) the mRNA attaches to the ribosome (acting as the platform for building the protein) and the corresponding tRNA with an attached amino acid approaches and drops the amino acid once it binds with the chain of amino acids, which are the new protein. (Modified from Lodish (2007), Martini (2006) and McArdle Katch & Katch (2007)).

2.2.3 The Cytoplasm

Protein translation occurs in the cytoplasm. Translation is a delicate process involving ribosomes, tRNA, mRNA and amino acids. A ribosome becomes active when a strand of mRNA binds with the small and large subunit of the ribosome and the transfer RNA (tRNA). Transfer RNA works closely with the mRNA so that the right amino acids bind in the correct order, and thus produce the correct protein. The mRNA passes through the ribosome attracting the tRNA that carries the correct amino acid that binds to the string of amino acids, eventually producing the protein. This continues until the end of the
mRNA is reached, and the string of amino acids is complete, and so too the protein (Lodish et al., 2007). Therefore, an increase in either transcription or translation can lead to an increase in protein synthesis and muscle mass. Understanding the factors that influence each of these is therefore important for understanding the best way to increase protein synthesis and therefore muscle mass.

2.3 Cytokines

2.3.1 Role of Cytokines

When infection or tissue damage occurs, a local production and release of cytokines occurs at the site of the inflammation (Booth & Neufer, 2006). Cytokines are polypeptides that were thought as having solely an immunoregulatory role (Febbraio & Pedersen, 2002; Fischer, 2006). However, it has recently been found that there is also an inflammatory response after exercise in a range of different populations (Abramson & Vaccarino, 2002; Buford, Cooke, Shelmadine et al., 2009; Buford, Cooke, & Willoughby, 2009; Fischer, 2006; Frydelund-Larsen et al., 2007; Izquierdo et al., 2009; Keller et al., 2005; Mastorakos & Ilias, 2006; Miles, Pearson, Andring, Kidd, & Volpe, 2007; S. R. Ostrowski et al., 2005; Pedersen, Akerstrom, Nielsen, & Fischer, 2007; Suzuki et al., 2002; Troseid et al., 2004). Muscle damage associated with some types of exercise were seen as the stimulus for this upregulation of cytokines after exercise; however, inflammatory responses have been shown after exercise that is not associated with muscle damage (Chan, Carey, Watt, & Febbraio, 2004; Frydelund-Larsen et al., 2007; Sorichter et al., 2006; Wong, 1990). This has lead researchers to examine a hypothesis that there is a relationship between the inflammatory response to exercise and exercise induced protein synthesis and therefore muscle mass.

In skeletal muscle, a cytokine cascade allows the cytokine process to occur. The cytokines released in the initial part of the cascade are (named in order) TNF-α, IL-1β, IL-6, IL-1 receptor antagonist (IL-1ra), and soluble TNF-α receptors (sTNF-R) (Figure 2.2) (Booth & Neufer, 2006). All of these cytokines
are upregulated after exercise, and may contribute to the process of protein synthesis (Booth & Neufer, 2006).

![Muscle Contraction Diagram]

Figure 2.2 A simplified schematic of the signaling cascade resulting from muscle contraction, which leads to the initiation of protein synthesis.

2.3.2 Cytokines and Exercise

Cytokines play a role in the physiological adaptation to exercise. After exercise, increased expression of IL-6 are found in the bloodstream and in the working muscle (Buford, Cooke, Shelmadine et al., 2009; Izquierdo et al., 2009; Pedersen et al., 2007; Petersen & Pedersen, 2005; Steensberg et al., 2000). In accordance with the cytokine cascade (Figure 2.2) the high concentrations of IL-6 lead to high concentrations of IL-1ra (Dinarello, 1991) and IL-10 [Ostrowski, 1999 2000] in the bloodstream after a bout of exercise. IL-1ra and IL-10 are anti-inflammatory molecules (Booth & Neufer, 2006), and with this anti-inflammatory effect IL-6 works in an anti-inflammatory manner. TNF-α is not expressed during exercise (Petersen & Pedersen, 2005; Steensberg, Keller et al., 2002; Steensberg et al., 2000), in which its production is inhibited by IL-6 (Matthys, Mitera, Heremans, Van Damme, & Billiau, 1995; Mizuhara et al., 1994) and is inversely related to protein synthesis rates (Greiwe, Cheng, Rubin,
Yarasheski, & Semenkovich, 2001). This indicates that the relationship between IL-6 and TNF-α contributes to the cytokine effect on protein synthesis.

Another cytokine that may play an important role in muscle protein synthesis is IL-8, which is a downstream target of IL-6. It is strongly influenced by IL-6, and also shows a large upregulation after exercise (Akerstrom et al., 2005; Buford, Cooke, Shelmadine et al., 2009; Cappelli et al., 2009; Chan et al., 2004; Frydelund-Larsen et al., 2007; Nielsen & Pedersen, 2007; Nieman et al., 2006; Nieman et al., 2001). Studies show that while long durations of exercise, including running (Buford, Cooke, Shelmadine, et al., 2009) and cycling (Akerstrom et al., 2005; Chan et al., 2004; Nieman et al., 2006) stimulate IL-8 production in the muscle, plasma IL-8 is only upregulated after intensive, and often eccentric (damaging), exercise (Akerstrom et al., 2005; K. Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 2001). In a study where participants completed a one-hour cycle exercise, IL-6 and IL-8 mRNA were significantly elevated, while TNF-α and IL-15 were not (Chan et al., 2004). This shows the close relationship between IL-6 and IL-8 in the cytokine cascade. This relationship is supported by many recent studies in humans and animals (Akerstrom et al., 2005; Buford, Cooke, Shelmadine et al., 2009; Cappelli et al., 2009; Chan et al., 2004; Frydelund-Larsen et al., 2007; Nielsen & Pedersen, 2007; Nieman et al., 2006; Peake et al., 2008).

As well as the acute effects of IL-6 and IL-8, there is support for a long-term effect of exercise on the inflammatory response. In the elderly, there are higher levels of the TNF-α protein and mRNA when compared to young men and women. This elevated level of TNF-α mRNA and protein reduced after 3 months of resistance exercise, and inversely related to the rate of muscle protein synthesis in the exercise group (Greiwe et al., 2001). This suggests that TNF-α is a contributor in the age-related decline in muscle mass, and that resistance training may help to slow this muscle wasting process by suppressing skeletal muscle TNF-α expression. From these findings, as well as those discussed earlier in this review, it can be extrapolated that there is a link between protein synthesis and the levels of IL-6 in the bloodstream and in
muscle that may be useful in the search for ways in which to prevent many chronic diseases.

2.3.3 The Link between Cytokines and Protein Synthesis/Degradation

Two of the most studied cytokines are IL-6 and IL-8, due to their relatively large changes in concentration and production in plasma and muscle, respectively. Haddad, Zaldivar, Cooper, & Adams (2005) injected IL-6 into the tibialis anterior of mice at levels similar to those found post-exercise. The injection of IL-6, without related systemic changes occurring, resulted in muscle atrophy (loss of myofibrillar protein of -17%) and a decrease in the phosphorylation of ribosomal S6 kinase (-60%) and STAT5 (-33%) and a twofold increase in STAT3. There were also increases in IGF-1 and SOCS-3, both of which are associated with muscle hypertrophy, indicating that there may be some relationship between the breakdown in muscle tissue and the signalling of hypertrophic related genes.

Two pathways that IL-6 appears to influence are the growth hormone (GH)-insulin-like growth factor (IGF)-1 axis and the Janus kinase (JAK)-signal transducer activator of transcription (STAT) pathway. The GH-IGF-1 axis plays a large role in the growth and adaptation of skeletal muscle (Haddad et al., 2005). IGF-1, directly stimulated by GH regulates some aspects of the adaptation of skeletal muscle and muscle growth and development (Adams & McCue, 1998; Haddad et al., 2005; Miyazaki & Esser, 2009). In addition, elevated levels of GH increase the development of skeletal muscle (Izquierdo et al., 2009; Miyazaki & Esser, 2009).

One of the roles of the JAK-STAT pathway is the translocation of STAT to the nucleus, to initiate the transcription and expression of a number of proteins. This second pathway is negatively regulated by suppressors of cytokine signalling (SOCS), and therefore affects STAT activity (Haddad et al., 2005). As cytokines including IL-6 influence SOCS there is some evidence of a link between IL-6, and other cytokines, to not only muscle atrophy, but also muscle hypertrophy.
It is more likely, however that IL-6 and its close relative, IL-8 more closely relate to the protein synthesis process than first thought. It is now understood that IL-6 and IL-8 are produced in the muscle, and their expression during exercise outweighs the concentrations seen in the plasma after exercise (Akerstrom et al., 2005; Chan, Carey, Watt, & Febbraio, 2004; Fischer, 2006; Nieman et al., 2003; Steensberg et al., 2000). This lead to the hypothesis that IL-6 and IL-8 have roles within the muscle that is evident without the occurrence of muscle damage.

IL-6 is part of the IL-6 family of cytokines (Pedersen, Akerstrom, Nielsen, & Fischer, 2007), which includes many of the cytokines mentioned in the preceding paragraphs of this section. There is an increase in plasma IL-6 concentration after prolonged exercise, without muscle damage. Generally, IL-6 gene expression increases exponentially during exercise, dependant on intensity, duration and the muscle mass involved in the exercise, and decreases during the recovery phase after the exercise (Febbraio & Pedersen, 2002; Klein, 1993; Suzuki et al., 2002). IL-6 mRNA as well as protein expression is upregulated in the muscle after exercise (Hiscock, Chan, Bisucci, Darby, & Febbraio, 2004) and IL-6 is released from the muscle during exercise (Steensberg, Keller, et al., 2002; Steensberg et al., 2000). Therefore, IL-6 increases in the bloodstream and in the muscle in its various forms, regardless of the muscle damage caused, but is still dependant on the intensity, duration and muscle mass that is involved in the exercise.

IL-8 is a part of the CXC family of cytokines (Nielsen & Pedersen, 2007; Pedersen et al., 2007). IL-8 is a chemokine that regulates neutrophils, an essential part of the innate immune system, but also plays an angiogenic role (Pedersen et al., 2007). IL-8 has been shown to respond to exercise, however the response is only seen after exhaustive exercise (Nieman et al., 2003; Nieman et al., 2001; Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 2001; Suzuki et al., 2003). These studies examined the effects of eccentric muscle contractions, and the plasma concentration of IL-8 was elevated as a result. In concentric exercise, such as rowing (Henson et al., 2000) or cycling (Chan et
al., 2004) plasma concentrations did not increase, and so it appears that IL-8 responds to muscle damage.

Recent research has examined the levels of IL-8 expression in the muscle. After treadmill running (Nieman et al., 2003) and cycle ergometer (Chan et al., 2004) exercise IL-8 mRNA was expressed without an increase in plasma IL-8 (Chan et al., 2004). Even in response to concentric exercise, there was an increase in the mRNA of IL-8 (Akerstrom et al., 2005). This suggests that production of IL-8 occurs in the muscle, and many have a role within the muscle that far exceeds the need for it outside of the muscle fibre. The function of IL-8 is still widely unknown, and while its expression locally, as opposed to systemically, suggests that it works in an endocrine or paracrine fashion, It is likely that IL-8 is involved in the process of angiogenesis within the muscle (Pedersen et al., 2007), strengthening the possible link between the cytokines and muscle mass.

2.4 Inflammatory Response to Exercise

2.4.1 Cytokines and the Inflammatory Response

The cytokine family consists of polypeptide regulators that are responsible for immune regulation and metabolic function, among other functions. Cytokines are profoundly different from hormones when discovered, however they may play a similar and synergistic role to hormones. The main feature that differentiates them is that while specific glands secrete hormones, many organs or any nucleated cell can secrete cytokines. Importantly, muscles, as multi-nucleated organs, can secrete cytokines (myokines). The fact that muscle secretes these myokines may help to explain the close relationship that exists between myokine release (e.g. IL-6 and IL-8 concentration) and exercise performance. Three hours after the commencement of exercise there was a substantial increase in plasma IL-6 concentration, with a peak of around a 19-fold increase. The IL-6 expression in muscle follows the same pattern as the plasma values (Steensberg et al., 2000). This relationship explains the role of IL-6 in maintaining glucose homeostasis in the muscle during long duration
exercise. The muscle may release the myokines into the bloodstream as a way of communicating the stress put on the muscle, initiating an influx of nutrients into the muscle. As well as being part of the protein degradation pathways myokines are also involved in the development and functioning of the immune system, but this review will not discuss this role.

### 2.4.2 Process of Protein Degradation

While muscle protein synthesis is required for muscle hypertrophy, protein degradation, also mediated by inflammatory processes, lead to muscle atrophy. However, the inflammatory processes may help to initiate the protein synthesis process. There has been little evidence of the link between the immune response and muscle protein synthesis. Exercise is known to decrease systemic inflammation (Abramson & Vaccarino, 2002; Bruunsgaard et al., 2003; Campbell et al., 2009; Geffken et al., 2001; King, Carek, Mainous, & Pearson, 2003; Lira et al., 2009; Petersen & Pedersen, 2005; Troseid et al., 2004) but more importantly, acute exercise bouts lead to an increase in localised inflammation (Bolster et al., 2003; Cappelli et al., 2009; Izquierdo et al., 2009; Keller et al., 2005; Louis, Raue, Yang, Jemiolo, & Trappe, 2007; Sorichter et al., 2006). Knowing that the contractile protein synthesis pathways, such as the Akt/mTOR pathway are upregulated within a few hours of the completion of exercise, it is thought that these lead to protein synthesis (Bodine et al., 2001; Bolster et al., 2003; Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009; Kubica, Bolster, Farrell, Kimball, & Jefferson, 2005; Leger et al., 2006; Parkington, LeBrasseur, Siebert, & Fielding, 2004). Thus, a link exists between inflammation and the processes of muscle building.

### 2.4.3 Inflammatory Markers and Exercise

Inflammatory markers expressed after heavy resistance exercise have been seen to be upregulated as much as 128 fold from rest levels (Fischer, 2006). Muscle damage from exercise was first associated with this huge response. However, recent research has found a large upregulation after
Concentric-only exercise, which does not cause muscle damage, unlike eccentric exercise (Booth & Neufer, 2006).

Concentric-only exercise does not elicit any change in markers of muscle damage, and so there is reason to believe that the upregulation of the inflammatory genes associated with concentric-only exercise is not in response to muscle damage (Frydelund-Larsen et al., 2007; Sorichter et al., 2006). Recently, Sorichter and colleagues (2006) found that there was a greater concentration of IL-6 within 24 hours after concentric-only exercise (60min run). When the same participants returned two weeks later, they completed an eccentric exercise session targeting the quadriceps femoris muscle, where IL-6 concentration did not increase after the exercise. This study provides important evidence that cytokine release and the inflammatory response are not only a specific response to muscle damage, but that they have an important role in the protein synthesis process.

2.5 Gene Expression

2.5.1 Gene Expression Pathways

A number of gene expression pathways that influence the rate of protein synthesis are between transcription and translation. These pathways contain a number of signalling molecules and proteins that, depending on their concentration increase or decrease the rate of the process (Boonyarom & Inui, 2006). One of the important pathways that influence protein synthesis in skeletal muscle is the mammalian target of rapamycin (mTOR) pathway.

The mTOR pathway is so called because one of the central signalling molecules in the pathway is mTOR (Leger et al., 2006). When mTOR is upregulated protein translation usually ensues (Booth & Neufer, 2006). There are a number of molecules involved in the mTOR pathway, as seen in Figure 2.3. There must be a balance between the activities of all the molecules in order for protein synthesis to occur. To initiate pathways such as mTOR, there must be a stimulus of some kind, such as a muscle contraction (Drummond et al.,
2009; Drummond & Rasmussen, 2008; Kubica et al., 2005; Leger et al., 2006; Parkington et al., 2004). Insulin, and glucose or leucine, (derived from nutrient ingestion) play a large part in the regulation of the mTOR pathway, and can influence the rate of protein synthesis (Adams & McCue, 1998; Drummond et al., 2009; Drummond & Rasmussen, 2008; Keller et al., 2001; Steensberg, van Hall, et al., 2002). Therefore, without appropriate nutrition, protein synthesis does not occur, resulting in the synthesis/degradation balance leaning towards the degradation.

While we know that these pathways are involved in the process of protein synthesis, other factors, such as cytokines IL-6 and IL-8 are upregulated with exercise (Chan et al., 2004; Nielsen & Pedersen, 2007; Nieman et al., 2003; Nieman et al., 2001; Suzuki et al., 2003; Timmons, Tarnopolsky, Snider, & Bar-Or, 2006), leading to an increased protein synthesis. There has not yet been any evidence, to our knowledge to suggest that IL-6 and IL-8 are related to the protein synthesis process, although suspicion arises from evidence showing that muscle can produce these cytokines (Hiscock et al., 2004; Pedersen et al., 2004; Pedersen et al., 2001; Plomgaard, Penkowa, & Pedersen, 2005). It is more likely that IL-6 and IL-8 act at the muscle level, as there have been no significant increases in their plasma concentration.

![Figure 2.3 A simplified schematic of the mTOR pathway, from the stimulus to protein elongation and transcription initiation.](image)

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2.6 Conclusion

Muscle mass is an important factor influencing the performance of activities of daily living and the performance of many sporting activities. The balance between protein synthesis and degradation determines the size of a muscle. Inflammatory pathways are important in protein synthesis, not just, as first thought in degradation. Cytokines, in the inflammatory pathways, upregulate as a response to exercise, and were thought to be as a response to the damage that can occur. Now some cytokines are seen as a prerequisite to protein synthesis. Two such cytokines that upregulate after exercise, IL-6 and IL-8 activity are seen to have a link to an increase in muscle mass after exercise.

Initially the link between upregulation of the cytokines and exercise was to their role in the repair of muscle damage however, the cytokines also up regulate after concentric exercises that do not cause muscle damage. In plasma, IL-8 concentration rarely upregulates after concentric exercise, but is upregulated in the muscle and therefore must provide a role other than that in muscle degradation. Now that IL-8, and IL-6 may both have endocrine or paracrine functions in muscle, research must be conducted to investigated whether a relationship exists between these as well as other genes and muscle protein synthesis.
CHAPTER THREE

Methodology
3.1 Overview

All participants attended the laboratory or gym up to two times a week for 10 weeks. The first two weeks were testing weeks involving, computed tomography (CT) scans, training familiarisation and the pre-training muscle biopsy sampling. Eight weeks of training followed, and post-training CT scans taken at the end of the 8 weeks of training (Figure 3.1).

![Figure 3.1. An overview of the testing protocol. Muscle biopsies (M. biop.) were taken at various time-points (immediately before and 1 hour after exercise) on the testing day in week three. Computerised Tomography (CT) scans were taken prior to the first testing session, and less than a week after the completion of the last session of the 8 weeks of training. One repetition maximum (1RM) testing was conducted before, and after the first 4 weeks of training in order to set training loads, with DEXA scanning completed in week 1+2 to set the standardised breakfast given before testing in week 3.](image)

3.2 Participants

Fourteen healthy, young men volunteered for the study, of which only 11 participants had viable biopsy samples taken. Muscle mass and protein synthesis are known to be affected by a number of factors, which include age (Mazzeo et al., 1998), gender, the prevalence of muscle loading exercise (Gerdhem et al., 2003; Grund et al., 2001; Lee et al., 2008; Stenholm et al., 2008) and disease (Lee et al., 2008). Participants were of a homogeneous sample in order to eliminate the possible effects of these factors. Participants chosen were healthy, of similar age, height and weight, derived from dual-
energy x-ray absorptiometry (DEXA) scans taken of participants in the initial 2 weeks of testing, prior to the commencement of training. (See table 3.1), and completed similar amounts of exercise per week, as was stated by the volunteers. To achieve recruitment targets, of the 30 volunteers recruited, 14 matched the selection criteria. All participants read and signed a consent form, and were given the opportunity to ask any questions prior to the commencement of the testing. The Human Research Ethics Committee of Edith Cowan University approved all aspects of the present study.

Table 3.1 Anthropometric parameters of the participants as a whole and as groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>Lean Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate Resistance</td>
<td>20.5 ± 1.9</td>
<td>181.4 ± 10.3</td>
<td>81.1 ± 22.1</td>
<td>64.6 ± 12.9</td>
</tr>
<tr>
<td>Training Group (MR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Resistance</td>
<td>22.4 ± 5.2</td>
<td>177.5 ± 9.4</td>
<td>78.8 ± 8.6</td>
<td>61.8 ± 5.0</td>
</tr>
<tr>
<td>Training Group (HR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Participants</td>
<td>21.6 ± 4.1</td>
<td>179.2 ± 9.6</td>
<td>79.8 ± 15.1</td>
<td>63.0 ± 8.9</td>
</tr>
</tbody>
</table>

3.3 Testing Procedures

3.3.1 One repetition maximum (1RM) testing

On day 1, concentric one repetition maximum (1-RM; the maximum load that the subject can successfully lift once) was determined for the leg press (to 90° knee angle); leg extension and leg curl exercises (to full extension or flexion, respectively). Also on day one was familiarisation of the training protocol, prior to the 1RM testing, with low loads (20-50% 1 RM) applied and several repetitions completed, and acting as a warm-up for the 1-RM. After each successful repetition, the load was increased, with 1-2 minutes rest between repetitions (Astorino, Rohmann, & Firth, 2008; Levingera et al., 2009). This continued until the participant was unable to complete one full repetition with appropriate technique. The highest completed load was deemed as the 1-
RM, the 1-RM was always reached within 3-6 attempts (Levingera et al., 2009; McCurdy, Langford, Cline, Doscher, & Hoff, 2004).

3.3.2 Muscle tissue sampling

In week 3, at least five days after the familiarisation and 1RM testing, the participants attended the laboratory after an overnight fast. Participants consumed a standardised meal consisting of 1.12g CHO, 0.15 g protein and 0.07 g fat/kg lean body mass (derived from the DEXA scans taken of participants) at the laboratory. Reasoning behind this was to reduce the effects of nutrient ingestion on subject performance. Such nutrient effects can come from, protein/essential amino acid (Drummond et al., 2009; Drummond & Rasmussen, 2008; Elliot, Cree, Sanford, Wolfe, & Tipton, 2006; Karlsson et al., 2004), free fatty acids (Lang, 2006) and carbohydrates (Børshøe et al., 2004; Drummond et al., 2009). In addition, the participants were told to refrain from alcohol (Suter & Schutz, 2008) for at least 24 hours, and caffeine (Deldicque & Francaux, 2008; Tipton, Jeukendrup, & Hespel, 2007) and other stimulants for at least 6 hours prior to testing. After 90 min of rest the initial bout of exercise (at a training load that corresponds to participants training group, explained later) was completed.

Immediately before and 1 hour after this initial session muscle tissue samples were obtained using a 13g catheter, with three rotations sampling about 60-110mg of tissue each. At a depth of 2-cm and at 50% of the length of the quadriceps the sample was taken with a triggered muscle biopsy needle (14×10g disposable biopsy core instrument, Bard Biopsy Systems, UK). A 5% lidocaine anaesthetic cream prevented participants from pertaining to unnecessary pain. At least 30 mins prior to the scheduled biopsy the skin, lightly abraded at the site to remove the top layer of skin cells, to increase the effectiveness of the lidocaine scheduled biopsy to allow adequate absorption of the lidocaine cream had a liberally amount of cream applied. A plastic covering applied to the site prevented the evaporation of the active ingredients in the cream. The muscle samples were immediately frozen in liquid nitrogen and stored at -80°C for future analysis.
3.3.3 Muscle cross-sectional area

Computed tomography (CT) assessed the muscle cross-sectional area (CSA) of the quadriceps muscle before and after the eight weeks of training. A trained radiographer, who took a single slice image at 50% of femur length, coinciding with the biopsy site, performed the CT scanning. Muscle mass accumulation varies along the length of the lower limb (Blazevich, Gill, Bronks, & Newton, 2003; Hakkinen et al., 2001; Housh, Housh, Johnson, & Chu, 1992; Kumagai et al., 2000; Narici et al., 1996), so the biopsy site and the CSA measurement occurred at the same location along the limb, i.e. halfway between the greater trochanter and the lateral epicondyle. CSA was measured from these images by tracing around the quadriceps muscle using freely available image analysis software, which has been shown previously to be reliable (CV=0.78%) (Image J: National Institutes of Health, USA). Figure 3.2 shows one subject’s CT image, with the quadriceps traced around ready for analysis. Statistical analysis used an average of duplicate measurements for each CSA measurement.

Figure 3.2 Example of one subject’s CT scan with the quadriceps muscle traced around ready for analysis.
3.4 Exercise Protocol

Participants completed eight weeks of resistance training using the same exercises as used in the acute bout. Training consisted of two sessions per week under the supervision and encouragement of an appropriately trained professional, with at least two days rest between training days. The aim of the ST was to isolate the leg-extensor muscle group, bilateral leg press and knee extension exercises. In order to prevent muscle imbalances the leg curl exercise was included in the training. Each of the three exercises required completion of three sets of either 6 or 10 repetitions (variable depending on the training group that participants were in, as explained below). Prior to all sessions a warm-up consisting of 5 min on a stationary cycle and lower leg stretches (hamstring, quadriceps, and gluts, held for up to 5 sec each leg, and repeated when participants felt muscle tightness), prior to each exercise, a reduced weight was used to perform warm-up sets. The warm-up was to minimise the risk of injury to the participants.

A high resistance training group (HR; n=8, average reps=6.3±0.5) performed the sessions at a load of 90% of a pre-determined 1-RM while a moderate-load resistance group (MR; n=7, average reps=10.0±0.0) trained at 70% of 1-RM. All subjects were trained at one of two intensities this was to act as pilot data for any further research in the area. By including the two intensities subject numbers were reduced however, the two intensities provided a better insight into the training intensity that would best suit a longer training study.

Throughout the training, maintenance of the rep range by varying training loads allowed for gains in strength, and were recorded for every session, i.e. once participants were able to complete the training load without a spotter's assistance, the weights were increased. After four weeks of training, 1RM was re-assessed, and the training loads recalculated. Heavy muscle loading of these magnitudes has been shown to result in significant upregulation of IL-6 and IL8 expression and protein synthesis (Steensberg et al., 2000). The differing loading that participants were given was to increase the variability of the CSA
changes, as some research has shown differences between different loading on muscle mass (Holm et al., 2008).

In order to aid the accumulation of muscle mass all participants consumed a protein drink (38.4 g protein, 3.8 g carbohydrate and 2.1 g fat per 100 kg lean mass, derived for DEXA scanning) made as per the manufacturer's instructions at the completion of each training session. Protein ingestion aids muscle mass accumulation (Cuthbertson et al., 2005; Elliot et al., 2006; Fujita et al., 2007; Volpi, Kobayashi, Sheffield-Moore, Mittendorfer, & Wolfe, 2003).

3.5 Analysis of muscle tissue

3.5.1 mRNA expression

Total RNA was extracted using a modification of the phenol/chloroform extraction and isopropanol precipitation protocol, using the ToTALLY RNA™ Kit (Ambion Inc., Austin, TX), as per the manufacturer's instructions. EDTA-treated water was used to suspend the RNA pellet. RNA quality and concentration were determined using the NanoDrop 1000 Spectrophotometer (ThermoScientific, Australia) following the manufacturer's instructions. Nuclease-free water (NFW) added diluted the sample to an appropriate concentration, and was then stored at -80°C.

3.5.2 cDNA synthesis

At 65°C, RNA was brought up to temperature and held for 10 min, followed by quenching on ice for 5 min before reverse transcription. Total RNA (1.0 µg) using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA), generated first-strand cDNA. RNA added to a master mix, containing 2x RT buffer and 20x Enzyme Mix was incubated at 37°C for 60 min using the PCR Express Thermal Cycler (Hybaid, Middlesex, UK). Termination of the reaction by incubation at 95°C for 5 min then held at 4°C for 5 min followed. cDNA was stored at -20°C for subsequent analysis.
3.5.3 Real-time PCR

The GenAmp 7500 sequence detection system (Applied Biosystems, Foster City, CA) was used to perform RT-PCR. Each sample underwent PCR in triplicate, with reaction volumes of 20μl, containing Power SYBR (Applied Biosystems, Foster City, CA), forward and reverse primers and cDNA template (diluted 1:20). Previous research has demonstrated that this method of triplication is reliable (CV=0.045%) (Shoemaker, Lorieau, Lau, Gillmor, & Palcic, 2005). To compensate for the variations in input RNA amounts and efficiency of RT, cyclophilin mRNA was quantified, and all results were normalised to these values. Fluorescent emission data were analysed for the critical threshold (Cr) values, with expression of the gene of interest normalised to cyclophilin and expressed as $2^{-\Delta CT}$. Primers were designed using Primer Express software package version 3.0 (Applied Biosystems, Foster City, CA) from the gene sequences obtained for the GenBank (See Appendix A for relevant primer sequences). Primer sequences were checked for specificity using BLAST (http://www.ncbi.nlm.gov/BLAST/) and then purchased from GeneWorks (Adelaide, SA, Australia).

3.6 Statistical Analysis

To account for the data not being normally distributed gene expression data was log transformed, and used throughout the correlation statistical analysis.

In order to determine if there was a relationship between the participants pre-training CSA and their gene expression response to training, Pearson's correlations were computed between the change in gene expression and the pre-training CSA. Secondly, to determine whether there was a relationship between the response of gene expression to training and increase in CSA of participants over the course of eight weeks of training, Pearson's correlations were computed between the change in gene expression over the exercise session and the change in CSA over the course of the training. Outliers (n=1) in the data were excluded from analysis if data points were greater than two
standard deviations from the mean. As data was not normally distributed, all
gene expression data was log transformed.

A Multivariate Analysis of Variance (MANOVA) was used to test for
between group differences in the changes in IL-6 and IL-8 expression. The
change in gene expression and CSA were analysed using multiple paired t-
tests. Significance was set at $p<0.05$

3.7 Testing Locations

Dedicated laboratories in Buildings 19 and 21 of Edith Cowan University,
Joondalup Campus hosted all strength training and testing, and muscle
sampling. CT scanning was performed at Envision Medical Imaging, Subiaco.
CHAPTER FOUR

Results
4.1 Study Participation

At the end of the study, there were 5 and 6 participants in the MR and HR groups respectively. Three participants did not have post-exercise samples taken, due to methodological issues.

4.2 Muscle Cross Sectional Area

Pre- and post-training CSA for both the MR and HR groups, as well as the pooled average of all participants is shown in Figure 4.1. There was no significant difference in CSA between the groups, either before or after the training ($p > 0.05$). Over the course of training, cross sectional area (CSA) increased in the MR group by $5.1\pm4.3\%$, and the HR group by $6.0\pm4.6\%$. Overall, there was a $6.0\pm4.2\%$ increase in CSA with the training. These increases were not shown to be significant.

![Graph showing cross sectional area increases](image)

Figure 4.1 Cross sectional area of the whole group (all), and the moderate resistance (MR) and high resistance (HR) groups before and after the eight weeks of training.

4.3 Gene Expression

A Multivariate Analysis of Variance (MANOVA) with repeated measures was completed for the two groups, MR and HR and over the two time points, pre-training and 1 hour post-training. There was a significant increase in IL-6 and IL-8 gene expression with the training ($p=.02$, and $p=0.04$, respectively). There was no significant between group differences in the responses to training.
groups for neither IL-6 nor IL-8 (p=0.36, and p=0.18, respectively), although there was a trend toward a greater response in MR (Figure 4.2).

![Graph a)](image1)

*a) Mean gene expression of IL-6 for MR and HR at pre- and post-exercise.

![Graph b)](image2)

*b) Mean gene expression of IL-8 for MR and HR at pre- and post-exercise.

Figure 4.2 a) Mean gene expression of IL-6 for MR and HR at pre- and post-exercise.
b) Mean gene expression of IL-8 for MR and HR at pre- and post-exercise.

In support of these findings IL-6 gene expression after exercise was 52-fold greater, with MR being 137 fold, and HR being 25 fold greater than rest values. IL-8 showed similar findings, 31-fold change for the whole group, then 129- fold change for MR and HR, respectively (Figure 4.3).
Figure 4.3 Change in IL-6 and IL-8 gene expression for all participants and both the MR and HR groups. The dotted columns represent the change in IL-6 for all participants combined and the two groups and the chequed columns represent the IL-8 change over the training session.

4.4 Muscle Cross Sectional Area and Gene Expression

4.4.1 Interleukin-6

With regard to the first research question, it was shown that the change in IL-6 expression was correlated with the pre-training CSA ($r=0.61$, $p$ (one tailed) < 0.05) (Figure 4.4a). The pre-training CSA was significantly related to the post-exercise IL-6 levels ($r=0.55$, $p$ (one tailed) < 0.05). With regard to the second research question, the change in CSA and change in IL-6 were not correlated, ($r=0.36$, $p$ (one tailed) > 0.05) (Figure 4.4b).
Figure 4.4 The raw change in IL-6 gene expression was correlated with a) pre- and b) change in cross-sectional area (CSA) of the quadriceps muscles for all participants.

For the MR group pre-training CSA was correlated with the change in IL-6 expression ($r=.93$, $p$ (one tailed) < 0.05). For HR the change in CSA was not correlated with the change in IL-6 expression ($r=.65$, $p$ (one tailed) > 0.05). Pre-training CSA and the change in IL-6 expression were not correlated ($r=-.17$, $p$ (one tailed) >0.05).

4.4.2 *Interleukin-8*

With regard to the first research question, it was shown that the change in IL-8 expression was correlated to the pre-training CSA ($r=.85$, $p$ (one tailed) <
In accordance with the second research question, the change in CSA and change in IL-8 were not correlated ($r = .12, p \text{ (one tailed)} > .05$) (Figure 4.5b).

![Diagram](image)

**Figure 4.5** The raw change in IL-8 gene expression correlated with a) pre- and b) change in cross-sectional Area (CSA) of the quadriceps muscles for all participants.

For the MR group pre-training CSA was correlated with the change in IL-8 expression ($r = .90, p \text{ (one tailed)} < .05$), where as the change in CSA and change in IL-8 expression was not correlated ($r = .61, p \text{ (one tailed)} > .05$). For HR the change in CSA was not correlated with the change in IL-8 expression ($r = .03, p \text{ (one tailed)} > .05$). Pre-training CSA and the change in IL-8 expression were not correlated ($r = .63, p \text{ (one tailed)} > .05$).
CHAPTER FIVE

Discussion and Recommendations for Future Research
5.1 Discussion

The maintenance of muscle mass is important in order to perform activities of daily living and most athletic tasks, and can influence the quality of life. Clinically, muscle mass can help in the recovery of patients who have had a period of long-term bed rest (Lee et al., 2008), and in the elderly it is important to have adequate muscle mass in order to prevent falls, which is a primary risk factor for premature death (Barber, Muller, Whitehurst, & Hay, 2010). Muscle mass is also important in the healthy population as it can improve athletic performance and improve general quality of life.

Muscle mass plays such a pivotal role in many aspects of daily living and athletic performance with much research done to understand muscle hypertrophy and atrophy (Adams & McCue, 1998; Baar & Esser, 1999; Bickel et al., 2005; Bodine et al., 2001; Ennis et al., 2007; Heinemeier et al., 2006; Leger et al., 2006; Miyazaki & Esser, 2009; Parkington et al., 2004). Recently studies have focused on protein synthesis, an underlying process regulating hypertrophy (Baar & Esser, 1999; Bolster et al., 2003; Burry, Hawkins, & Spangenberg, 2007; Cheng, Fryer, Carling, & Shepherd, 2004; Dreyer et al., 2006; Ennis et al., 2007; Karlsson et al., 2004; Kubica et al., 2005; Leger et al., 2006; Mascher et al., 2008; Parkington et al., 2004; Spangenburg & McBride, 2006). A number of different pathways are involved in protein synthesis. Recent findings are beginning to uncover pathways and processes that may have a greater role than first thought, one of which is the inflammatory processes. Muscle damage was seen as a way to explain the upregulation of inflammatory processes after exercise (Miles et al., 2007; Silva et al., 2008). However, the inflammatory response may be a prerequisite to the protein synthesis pathways. Inflammatory gene expression is an important determinant of muscle mass production with the early inflammatory response that has been shown to be important in the process of protein synthesis (Furmanczyk & Quinn, 2003). Little research has investigated the relationship between these inflammatory pathways and protein synthesis, let alone with muscle mass. In the present study two of the many inflammatory genes expressed after exercise were investigated and the relationship with cross-sectional area was examined.
With regards to the first research question, it was shown that there is a relationship between the change in IL-6 and IL-8 expression after a bout of RE and the initial CSA of participants. This indicates that there may be a relationship between inflammatory gene expression and muscle mass. This supports the hypothesis that participants with a larger muscle mass have a greater inflammatory gene expression response, and therefore can theoretically have a greater muscle mass accumulation with exercise. Because IL-6 and IL-8 upregulation is thought to be related to protein synthesis this may explain this link between IL-6 and IL-8 upregulation and muscle mass. In the present study it was found that while inflammatory gene expression up-regulates quickly after exercise, where as protein synthesis genes, such as 70-kDa S6 protein kinase (p70S6k) have been shown to up-regulate later in the recovery phase (Baar & Esser, 1999; Burry et al., 2007). This suggests that while p70S6k is important in protein synthesis, it may become clear that inflammatory genes may play a larger role in the process than initially thought.

In accordance with the second research question, it was shown that the increase in CSA seen after the eight weeks of training was not related to the change in gene expression of IL-6 nor IL-8 after a single exercise bout. In other words, those with a larger muscle mass after the training did not necessarily have the larger inflammatory response to the exercise. The relationship between change in CSA or muscle mass and inflammatory gene expression has not been examined, to our knowledge until now, so definite conclusions are yet to be drawn. It may be possible that there is a 'U-shaped' relationship between gene expression and CSA, as seen in Figure 4.4 between the change in IL-6 and the change in CSA as well as with the pre-training CSA. More research needs to be done to support this theory. Evidence suggests that inflammatory gene expression has negative effects on protein synthesis processes, and therefore muscle mass (Nielsen & Pedersen, 2007). However, the expression of inflammatory genes, such as IL-6 has been shown to upregulate after exercise shown not to cause the damage that leads to the negative inflammatory response (Chan et al., 2004; Sorichter et al., 2006). This shows that the although it was originally thought that inflammatory gene
expression was related to the recovery from damaging exercise, they may in fact play a role in the protein synthesis processes, and so the present study was completed in an attempt to support this theory.

All but one of the correlations, when the groups were correlated separately were similar. The MR group showed a correlation between post-exercise IL-6 as well as IL-8 expression and pre-training CSA. This suggests that in the MR group those with the biggest expression after the single exercise bout were those that had the largest muscle mass to begin with. This finding supports the hypothesis that those that have the largest expression of inflammatory genes will have the largest muscle mass, and therefore could lead to a greater gain in muscle mass. Protein synthesis molecules, such as p70^{S6k} have also been seen to be phosphorylated in large quantities in the muscle after a range of exercises, including resistance exercise known to induce muscle hypertrophy (Baar & Esser, 1999; Burry et al., 2007; Karlsson et al., 2004). It was seen that the type (Burry et al., 2007; Sorichter et al., 2006) or intensity of the training influenced the expression of many of these molecules (Ostrowski et al., 2000).

5.2 Limitations and Assumptions

The limitations of this study include the ability to control the physical activity of the participants; all participants were encouraged to keep activity levels the same throughout the study.

In addition, the small sample sizes used in the present study could help to explain the lack of significant difference in the change in gene expression and change in CSA. While there were 11 participants trained as a part of this study, the two different training intensities lead to the group sizes being small, and may have lead to a lack of significant differences in the grouped data. The two training groups were included to act as a pilot to future research, as different exercise intensities lead to varying responses across a range of measures, including the magnitude of muscle mass accumulation (Holm et al., 2008; Ostrowski et al., 2000). In future research it would be beneficial to use larger
sample sizes, and there may be stronger relationships than shown in the present study.

While IL-6 and IL-8 were specifically chosen for the present research many other inflammatory genes are expressed after exercise and may have a significant influence on muscle mass. Only these two genes were chosen due to time and financial constraints that were imposed on the research.

5.3 Recommendations for Future Research

Young healthy men were examined in the present study, however this cannot be directly applied to aging or diseased populations The present research gives grounds for further examination of different populations, such as the elderly in order to find a more effective way to train for the prevention of falls, for example. It would be beneficial to examine other populations including aging and/or diseased populations on the relationship between gene expression and muscle mass. Not only other populations but also other inflammatory genes, and larger sample sizes are recommended for future research. It would be of interest to investigate genes such as IL-1α, IL-10 and TNF-α, among others at different time points that correspond with the greatest upregulation of these genes.

5.4 Conclusion

The present research shows that those with a larger muscle mass have a greater inflammatory response to an exercise bout. However, those that have large increases in gene expression over the course of a single exercise bout do not necessarily show a greater increase in muscle mass over the course of a period of strength training. Moderate intensity exercise leads to those with a larger change in muscle mass having a bigger inflammatory gene response to the training. So, it can be concluded that the greater the inflammatory response the larger muscle mass they tend to have, but not necessarily the more muscle mass they will build over the course of a period of resistance exercise.


APPENDIX A:

Primer Sequences used in PCR Analysis

<table>
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<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
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<td>NM_000584</td>
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</table>
Computed tomography (CT) is a medical imaging technique that produces a three-dimensional image of the inside of an object using many two-dimensional images taken around an axis of rotation. CT uses a method known as tomography, which is the process by which images are acquired by sections or segments, i.e. it takes an image of a 'slice' of the body segment, as in Figure 1. The source of the radiation rotates around the body segment, and emits a fan-shaped x-ray beam that passes through the body segment to x-ray detectors that rotate parallel and opposite to the source, on the far side of the segment, as is shown in Figure 2.

The first concept of CT was presented in the early 1900’s when it was proposed that a single slice of the body could be projected onto a photographic film. The 1970’s saw the production of the first CT scanner, and though funding from EMI (the music company) the scanner, then called the EMI scanner, was developed for clinical use.

Whist CT is still an expensive medical imaging tool, it is considered the gold standard when diagnosing a number of different disease entities, costing anywhere up to $500 per scan when produced commercially. CT can scan any part of the body, and there are few possible side effects from the scan itself (although it has been found that the intravenously administered contrast agents sometimes used to gain a better image can cause some adverse effects; in the present study no contrast agents will be used).

One of the main concerns with many scans is the dose of x-ray radiation that is used to perform the scan. Since the higher the dose the better the quality of the image and the lower the dose the more noise and blurring that can occur, a sacrifice must be made so that the lowest possible dose is given without jeopardising the quality of the image. To avoid this, computer programs are used to filter out noise and enhance structures, also different body parts require different amounts of radiation to produce an image, so the dose can be individualised to the body part.

To perform the scan, x-ray slice data is generated using an x-ray source that rotates around the object; x-ray sensors are positioned on the opposite side of the circle from the x-ray source. Subjects lie on a table and the area to be scanned is positioned under the scanner. The subject then remains as still as possible to avoid blurring of the image. The scan takes approximately 10 minutes to complete.
Micro-Biopsy Muscle Sampling Techniques

Biopsies have been used for many years in medicine to allow the sampling of tissues to help diagnose many pathologies of the nervous, vascular and musculoskeletal systems, as well as connective tissues and visceral organs. Individuals with myopathies resulting in low muscle mass and strength (i.e. problems with the muscle itself) and neuropathies (problems with the nerves innervating the muscle) have been diagnosed with the use of biopsies in recent years.

In sports science research, muscle biopsies have enabled the study of fibre types and the concentrations of a number of different chemicals, proteins and enzymes that the muscle produces as a response to exercise. In more recent times muscle biopsies have been a useful tool in medicine and in sport science research as a tool in assessing the genetic make-up of a muscle. In medicine, interest lies in predicting of the likelihood of someone developing any one of a number of diseases. In sports science, muscle biopsies can be useful in assessing the different genes that are related to different aspects of the physiological response to training, such as hypertrophy.

There are two types of muscle biopsy, the standard and the micro-biopsy. Micro-biopsies are done by inserting a needle directly into the muscle to remove a small part of the tissue, or alternatively through a surgical incision, as in the standard biopsy technique. Both of the procedures are considered invasive, however the former is less invasive and therefore more comfortable for the patient. In the former technique (micro-biopsy) a needle is inserted into the muscle of choice, commonly the vastus lateralis, to remove a small sample of tissue. Usually 40 mg of muscle tissue will be taken, as opposed to the 180 mg taken during the traditional biopsy methods. It is common practice to also use an anaesthetic cream to numb the skin before the muscle biopsy is done. Subjects will often only feel slight pressure in the muscle, which has been described as 2-3 on a scale of 1-10.

The risks associated with this micro-biopsy procedure are almost nil, and even lower than with the traditional biopsy as there is no wound management required and thus a reduced chance of infection. Both traditional and micro-biopsy techniques are safe when performed by an appropriately trained researcher, as will be done in the present study.
APPENDIX C:

Participants Recruitment Letter (distributed by email)

I hope that you did well in all your exams. This email is to let you know a bit more about the exciting opportunity to participate in the world-leading research that we are conducting in semester 2 of this year.

Strength training programs are often given to increase muscle strength and size in normal, athletic or clinical populations. A number of training parameters (e.g. the load lifted, speeds of movement, range of motion) can be altered with differing effects on strength and size. However, it is not clear what combination of parameters is optimum for different populations.

Longitudinal (e.g. 6 - 52 weeks) training studies can be performed to ascertain the effects of training with a given set of parameters in a specific population, but the studies take so long to complete the use of longitudinal studies ensures that our understanding progresses slowly. More recently it has become possible to measure the activities of certain genes that are 'switched on' when muscle mass is gained (or 'switched off' when muscle mass is lost). So it is becoming increasingly common to measure the activities of certain genes after individuals perform a single bout of strength training.

Researchers assume that the increases in gene activities are correlated with the long-term increases in size and strength that would have occurred if the training had been continued. Unfortunately it is still not clear which genes are best to study. Also, the accuracy with which we can predict the long-term outcome of training from gene activities has not been examined in humans, so we really don’t know whether this ‘genetic’ approach is effective.

In this first study in a program of research, we will examine the relationship between the changes in activities of several key 'muscle building' genes and the increase in muscle size and strength after a prolonged (8-week) training block. We will also assess whether these genes show substantial differences in their activities when the training loads are varied slightly but the overall quantity of work done is constant (i.e. heavier vs. lighter training). The results are very important for our understanding of how gene activities influence long-term muscle adaptations.

In order to determine the gene expression levels we have teamed up with high level researchers from The University of NSW. We will use the latest muscle sampling and imaging techniques, including dual x-ray absorptiometry and computerised tomography (CT) and ultrasound scanning. We will also use techniques which allow a very small amount of muscle tissue to be taken and analysed for muscle protein levels and related gene expression levels. These levels will tell us your adaptive responses to the training performed.

As sport science students, you will be given an opportunity to participate and learn from leading scientists in the field. Some of the benefits to you are;

- The opportunity to participate in world-leading research
• The chance to gain knowledge about your own strength, muscle mass, protein synthesis response, and gene expression
• The opportunity to contribute to the advancement of sport science as a research participant
• The chance to learn about the latest muscle and strength testing technologies
• Being first to obtain the results from, and practical implications of the study

Unfortunately, we presently only have the funding to accept 16 subjects, although, given the interest in the study, we are hoping to secure further funding so that we can include some more.

In order to be a participant, it is important that you fit the following participation criteria:

1. You have not completed any resistance training on your legs over the last 6 months (and don’t intend to)
2. You perform only recreational level physical activity (4 or fewer times a week)
3. You are fit and healthy and are able to perform maximal exertion exercise

If you are chosen to participate, you will need to be available in between weeks 3 and 12 of semester 2, including the mid-semester break. Testing will be performed in weeks 3, 4, and 12. The training will be performed in weeks 5-11, three times a week, including the mid-semester break. Training will take about 20 minutes per session and be done in one of our research gyms here at the university.

Some of you might elect not to participate in the study itself but simply come to watch some of the testing being done.

I hope this email gives you a better idea of what we hope to achieve and what would be required of you as a participant. We will be in contact with those of you who are interested in this exciting opportunity prior to the commencement of next semester. In the meantime we will send you some information pages explaining our research techniques, so that you can learn more about them.

We look forward to talking to you about the study soon.

Enjoy your break!
APPENDIX D:
Information Letter to Participants and Informed Consent

FORM OF DISCLOSURE
AND INFORMED CONSENT

Does myogenic gene expression predict resistance training-induced muscle size and strength adaptations?

You are invited to participate in an exciting research project designed to determine whether it is possible to predict the muscle size changes caused by a prolonged period of strength training from muscle gene responses to a single bout of the training.

Introduction
The most effective way to improve muscle strength is to complete a strength training program. However, there are a variety of different loading patterns that can be used within a strength training program, in particular the load (i.e. intensity), movement speeds, movement ranges, and muscle contraction modes (static vs. dynamic) can be manipulated to varying effects. Therefore, an important objective of researchers is to identify the best loading patterns for different populations of individuals. However, there are nearly an infinite number of possible variations in these loading patterns so a nearly infinite number of research studies are required. Given that the responses to a specific program of strength training can only be assessed after several months of training, the process of determining ‘best programs’ is very slow.

One way to reduce the time required is to directly measure the activity of genes that are associated with muscle building rather than to wait until large enough changes in muscle size occur that they can be measured by medical imaging techniques (e.g. MRI scanning). A recently developed method to do this is to ask an individual to perform a single bout of training, obtain a very small piece of muscle tissue after training, and then measure the activity of genes that are important to the muscle-building process. By doing this we can compare the effects of different loading patterns in studies lasting weeks rather than months, and significantly accelerate the rate at which we learn about the effects of loading pattern variations.

However, there is one substantial drawback of this paradigm: It has not yet known which gene, or set of genes should be studied. Clearly, there is a need to find the genes whose activities change with a single bout of training and that are predictive of the longer-term muscle size changes. Thus, one purpose of the present research is to examine changes in several important genes after a single training bout and to examine the relationships between their changes and the overall muscle size change after 8 weeks of training.

Whilst there is evidence to suggest that ‘heavy’ resistance training (using loads >90% of maximum strength) provides a greater stimulus for muscle adaptation compared to lower intensity training (e.g. 70% maximum strength), there are no studies that have compared the acute and long term adaptations at a gene level to these two contrasting
training interventions. A second purpose of the study is, for the first time in humans, to examine both the acute and long-term gene responses of resistance exercise comprising either resistance training performed at 70 or 90% of each subject's maximum strength. The study marks an important step in understanding the influence of gene activities on muscle mass change in response to strength training.

**Methods**

In this study, we will target eight genes for study. These genes have been shown to be highly important signalling molecules for muscle protein synthesis or degradation in human skeletal muscle. We intend to study the response of these genes to a high-intensity strength training (ST) bout before and after you perform four weeks of either high- (90% of maximum) or moderate-intensity (70% of maximum) ST. ST will be completed twice a week and involve leg press, leg extension and leg curl exercises being performed for 3-4 sets each with each set being performed until fatigue. We will then examine the relationship between the change in gene activity in response to the high-intensity ST bouts (both before and after four weeks of training) and the change in muscle size after training for eight weeks.

**Subjects**

Sixteen healthy, young males without history of pathology or musculoskeletal injury will volunteer for the study. To participate, you will have to pass a pre-training screening process to ensure you have no health-related problems that would stop you performing maximal exercise. You must not have performed heavy ST in the 12 months prior to the study. You will be informed about the experimental design and the associated risks and discomforts, then sign an informed consent document. The study has been approved by the Edith Cowan University Ethics Committee (for more information, please visit: http://www.ecu.edu.au/GPPS/ethics/).

**Design**

The study design is shown in Figure 1. At the beginning of the study and after 4 weeks of training you will perform a single ST bout at a load of either 70% of one repetition maximum strength (1-RM; the maximum load that you can successfully lift once) or at 90% of 1-RM. A small sample of blood (ear lobe and forearm vein) and muscle tissue (vastus lateralis, i.e. lateral thigh muscle) will be obtained before and both 1 and 3 hours after the training bout. Blood and muscle tissue samples will also be taken 3 days after the final training session. You will complete 8 weeks of progressive ST performed twice per week with the loads you used during the acute bouts (i.e. either 70 or 90% of 1-RM). During the training period dietary intake will be monitored and you will be advised on normal nutritional intake for your size and energy requirements.
Pre-and post-training testing (muscle size and

Pre-exercise muscle and blood sample

Post-exercise muscle and blood samples (x2)

Exercise stimulus

Figure 1. Overview of study design. You will train for 8 weeks. At weeks 1 and 4 muscle and blood samples will be taken before and after an acute ST bout. Half of the subjects will lift a load equivalent to 70% of their 1-RM load and the other half will lift 90% of their 1-RM load. A single resting muscle and blood sample will also be taken 3 days after the final training session.

Muscle cross-sectional area and anthropometry
The muscle cross-sectional area (CSA) and volume of vastus lateralis of the right thigh muscle will be measured from scans obtained by computed tomography (CT) by a trained radiographer. Five CT pictures will be taken across the thigh. Total, lean and fat mass will be determined before and after the 8-week-period using dual x-ray absorptiometry (DEXA). This is a very low dose x-ray system that will take a single picture of your body and reveal your lean and fat mass.

One repetition maximum (1RM) testing
Before training and at week 4, maximum strength for leg press and leg extension exercises will be determined. After each successful repetition, the load will be increased until you are unable to complete one full repetition with appropriate technique. The highest completed load will be deemed as the 1-RM.

Static and dynamic muscle strength
Maximum knee extensor torque will be determined when the knee is allowed to extend at 30 and 300°·s⁻¹ when the leg is fixed to a motor-driven machine (isokinetic dynamometer). After warm-up and familiarisation, you will perform 3 and 5 continuous repetitions at the slow and fast speeds, respectively, with the aim of producing maximum knee extension torque. In addition, maximum isometric torque will be measured with the knee joint 60° from full extension.

Muscle tissue sampling
You will attend the laboratory after an overnight fast before consuming a standardised meal. After 90 min of rest you will complete a single bout of ST. Small muscle tissue samples will be obtained from the vastus lateralis (lateral thigh muscle) immediately before and 1 and 3 h after the ST session at 0 and 4 weeks of training. A resting sample will also be taken 3 days after the final training session.

To obtain the sample, we will use the minimally-invasive fine-needle or microbiopsy technique. In this procedure a hypodermic microbiopsy needle (14-gauge) is inserted into the muscle. Using a trigger mechanism, the needle takes a small (~20 mg) sample of tissue. A skin incision is not required (and thus no wound treatment is needed) and a hypodermic anaesthetic is not essential although a local anaesthetic cream will be applied so that the procedure is completely painless. A very small section of muscle is obtained. This technique has recently become common, partly because it carries an even smaller injury risk and considerably less discomfort than traditional muscle biopsy procedures. There is also no chance of scarring using the technique and the risk of infection is negligible as no incision is made in the skin.

Analysis of muscle tissue
We will apply standard tissue sample analysis procedures to determine gene activities in each sample of muscle tissue.

Blood sampling
Three blood samples will be obtained from a small (~2 mm) incision made to your earlobe for lactate analysis. Furthermore, an 8 ml blood sample will be obtained from your forearm vein (as per a normal hospital blood test) before and after the exercise. Collection of blood is a standard procedure conducted under sterile conditions and is associated with little discomfort or risk of infection.

Benefits to You
This is the first study of its type to be conducted at ECU and one of the few studies of its type to be conducted in Australia. As such, you, as a student in sport sciences, will be the first to experience these research techniques first hand, have the unique opportunity to learn about gene research and its possible future impact on sport sciences research, and have the opportunity to learn more about strength training and testing procedures from the qualified staff. Of course, you will also participate in research that will make a major contribution to our knowledge of strength and muscle mass adaptations to strength training.

Possible Risks and Discomforts
As with all maximum force testing and training, there a small chance of muscle strains. This risk will be further minimised by the use of full familiarisation of procedures, proper warm-up and practice repetitions prior to the maximal testing. All testing and training will be supervised by a researcher with considerable experience in muscle strength testing and training procedures.

DEXA scans are routinely used in clinical settings but carry a small risk to the patient. DEXA involves exposure to radiation. The radiation levels are exceedingly small (1-6 µSv) in comparison to the annual radiation western communities are naturally exposed to. To compare, a single chest x-ray would expose you to 30-40 µSv. The number of scans proposed in this study is well within the guidelines provided by the equipment manufacturer.

As with all invasive procedures, the muscle sampling procedure carries a very small chance of infection. This chance will be minimised by the use of alcohol swabs to clean the skin prior to needle insertion, and the use of sterile, disposable needles.
Unlike other muscle biopsy procedures, there is no need for an incision to be made in the skin with this procedure. The procedure is pain free and you will be able to use your muscles normally immediately after the needle is removed.

During the experiments venous blood will be collected from the forearm via a small needle. Bruising to the area where the blood will be collected may occur. There is also a small risk of infection, but this will be minimised using aseptic procedures. This is a standard procedure in this and other laboratories with no reported adverse reactions or outcomes. A trained technician, accredited with the procedure, will conduct the procedures.

Ear lobe blood samples will also be taken. This requires a small (~2mm) incision to be made in the ear lobe. A small (<0.1 ml) blood sample is taken. This procedure is very safe and has been conducted many times in our laboratory (and is commonly used to monitor training progress in athletes) with no recorded adverse reactions or outcomes. There is a small chance of itchiness on the ear, and the risk of infection is very low when appropriate procedures are followed.

**Investigator responsibilities**

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission.

If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice. However, prior notice of withdrawal would be appreciated.

If you have any questions, please contact:

Assoc Prof Anthony Blazevich  
Building 17.111, Joondalup Campus  
Edith Cowan University, Joondalup, 6027  
Western Australia

Phone: (08) 6304 5472  
Email: a.blazevich@ecu.edu.au
If you participate in the study and wish to talk to someone else about the project or your participation in it, please feel free to contact:

Prof Ken Nosaka, Head of Postgraduate Studies, School of Exercise, Biomedical and Health Sciences.
Room: 19.163 Joondalup Campus.
Phone: (08) 6304 5655.
Email: k.nosaka@ecu.edu.au.

OR

Kim Gifkins
Research Ethics Officer
Building 1, Block 'B', Level 3, Room 333, Joondalup Campus
Tel: (+61 8) 6304 2170
Email: research.ethics@ecu.edu.au

You will be given a copy of this form to keep.
Subject’s declaration of consent

I ___________________________, being over eighteen years of age consent to being a participant in the research project 'Differential protein synthesis and degradation in human skeletal muscle in response to strength training with different loading intensities'.

I have been given a copy of a 'Form of Disclosure and Informed Consent' document that I fully understand, describing the procedures to be followed and the consequences and risks involved in my participation as a subject. I am aware that blood and tissue samples will be taken, and understand the risks associated with these procedures.

I have read the information above and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw from the study without prejudice at any time.

I agree that research data gathered from the study may be published provided my name is not used. This includes data regarding your gene response to the exercise. This gene data is not sufficient to identify illness/disease, family lineage, etc.

Name of subject ____________________________
Signature of subject ____________________________ Date _______

Name of witness ____________________________
Signature of witness ____________________________ Date _______
Signature of researcher ____________________________ Date _______

Certifying that the terms of the form have been verbally explained to the subject, that the subject appears to understand the terms prior to signing the form, and that proper arrangements have been made for an interpreter where English is not the subject’s first language.
APPENDIX E:

Physical Activity Readiness Questionnaire - PAR-Q
(Revised 1994)

PAR-Q & YOU
(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active then you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common Sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

Yes  No
☐  ☐  1. Has your doctor ever said you have heart trouble?
☐  ☐  2. Do you frequently have pains in your heart and chest?
☐  ☐  3. Do you often feel faint or have spells of severe dizziness?
☐  ☐  4. Has a doctor ever told you that you have a bone or joint problem such as arthritis that has been aggravated by exercise, or might be made worse with exercise?
☐  ☐  5. Is there a good physical reason not mentioned here why you should not follow an activity program even if you wanted to?
☐  ☐  6. Are you over age 65 and not accustomed to vigorous exercise?

IF YOU ANSWERED YES to one or more questions
If you have not recently done so, consult with your personal physician by telephone or in person before increasing your physical activity and/or taking a fitness test.

NO to all questions
If you answered PAR-Q accurately, you have reasonable assurance of your present suitability for exercise.

DELAY BECOMING MUCH MORE ACTIVE
"If you are not feeling well because of a temporary illness such as a cold or a fever -- wait until you feel better; or "If you are or may be pregnant -- talk to your doctor before you start becoming more active.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology Health Canada and their agents assume no liability for persons who undertake physical activity, and in doubt after completing this questionnaire, consult your doctor prior to physical activity.

You are encouraged to copy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

I HAVE READ, UNDERSTOOD AND COMPLETED THIS QUESTIONNAIRE. ANY QUESTIONS I HAD WERE ANSWERED TO MY FULL SATISFACTION.

Name (Please print): ___________________ Signature: ___________________
(Must be 18 years or older)

Page: 68
APPENDIX F:
Subject Questionnaire

Name: __________________

Training Availability:
Colour in the grid, with you times you are unavailable, available, and times you are available but would rather not train by following the key below.

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unavailable | available | Available/rather not train

Personal Preference Information

As a part of the study, there is a requirement for a nutritional input on testing days and training days. So it must be asked, can you and would you drink plain milk,

and, can you consume milk with cereal (crunchy nut cornflakes)?

Are you available to train in the mid-semester break?

Do you have any labs, or practical assessments in the testing weeks, i.e. weeks 4, 9 and 12, that could effect your participation in testing and for what classes? (arrangements may be able to be made round this)