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In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.
MOLECULAR AND NEUROMUSCULAR RESPONSES AND ADAPTATIONS TO HIGH-POWER, HIGH-INTENSITY RESISTANCE EXERCISE IN COMPETITIVE WEIGHTLIFTERS AND RESISTANCE TRAINED ADULTS

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ABSTRACT

The sport of weightlifting requires the body weight-categorized athletes to perform three attempts of the snatch and three attempts of the clean and jerk in competition. During the performance of these lifts, competitive weightlifters have achieved some of the highest absolute and relative peak power outputs reported in the literature. The training methods of these athletes exceed evidence-based recommendations for the improvement of strength and power in resistance-trained adults. Thus, the purpose of this thesis was to identify and compare acute molecular and neuromuscular responses during the early recovery from high-power, high-intensity resistance exercise (HIRE) in competitive weightlifters and resistance-trained adults. An integrative physiological approach was taken across three separate research projects, with measurements of mRNA and protein expression changes made at the systemic and skeletal muscle level. In addition, performance tests were conducted along with direct quantification of skeletal muscle structural and functional responses following repeated HIRE in competitive weightlifters and resistance-trained adults. Of specific interest were the influence of prescribed variations in training load (i.e. 2 wk of overload and 1 wk of recovery training; Study 1) and the effects of post-exercise feeding (Study 3) on the exercise-induced transcriptional responses in competitive weightlifters and resistance-trained adults, respectively. In addition, as competitive weightlifters routinely perform multiple HIRE sessions within the same day, the skeletal muscle structural and functional responses to “double-day training” were compared between competitive weightlifters and resistance-trained adults (Study 2). The results presented in this thesis demonstrate that competitive weightlifters have a unique ability to recover rapidly following acute periods of overload and between successive HIRE sessions performed within the same day. In addition, a meal following an acute bout of high-power, HIRE was shown to have an attenuating effect on the expression of chemokines (MCP-1, CCR2) associated with muscle repair and regeneration. However, further research is required to determine the functional relevance of this exercise-nutrient interaction on athletic performance. Independently and collectively the findings presented in this thesis contribute to the understanding of the acute molecular and/or neuromuscular responses in well-trained adults following high-power, HIRE that lead to enhanced recovery and performance.
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STATEMENT OF CONTRIBUTION

In addition to the experiments presented in this thesis, I contributed to the following work during the course of study:

- Co-supervised Samantha Wong’s 2010 Health Research Council Summer Studentship (“Post Activation Potentiation – Acute changes in skeletal muscle structure and function following two successive bouts of high intensity resistance exercise”).

- Co-supervised Samantha Wong’s 2011 Faculty of Science Summer Studentship (“Systemic ‘stress-related’ responses to intense muscular exercise and feeding”).

- Participated in experimental design and sample collection for Stefan Wette’s Masters Thesis; “Molecular Responses to Power Resistance Exercise and Feeding in Resistance Trained Men”.

LIST OF PUBLICATIONS

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>1RM</td>
<td>1 repetition maximum</td>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<td>CCL4</td>
<td>chemokine (C-C motif) ligand 4</td>
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<td>CI</td>
<td>contractile impulse</td>
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<td>CK</td>
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<td>C&lt;sub&gt;t&lt;/sub&gt;</td>
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<td>DALDA</td>
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<td>DDIT4</td>
<td>DNA-damage inducible transcript 4 protein</td>
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<td>dH&lt;sub&gt;2&lt;/sub&gt;O</td>
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<td>DOMS</td>
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<td>GAPDH</td>
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<td>HIRE</td>
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<td>mg</td>
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<td>MIP-1β</td>
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<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
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<td>PC</td>
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<td>Profile of Mood States</td>
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<td>qPCR</td>
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<td>TMDS</td>
<td>total mood disturbance score</td>
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1.0 INTRODUCTION
Background

Weightlifting is a dynamic strength and power sport in which two, multi-joint, whole-body lifts are performed in competition; the snatch and the clean and jerk. During the performance of these lifts in competition, weightlifters have achieved some of the highest absolute and relative peak power outputs reported in the literature (Garhammer, 1980, 1982, 1991, 1993). The estimated power outputs reported in these previous investigations were determined by motion capture analysis in which the positions of the barbell during the performance of the lifts were digitized from projected film at specified time intervals (e.g. 0.04 sec). Barbell velocities and accelerations were obtained from the position-time data and were used in the estimation of power output. The training methods of these athletes are characterized by the frequent use of multi-joint, high-power, high-intensity resistance exercises (HIRE) in addition to the performance of the competitive lifts. For example, International-level weightlifters are known to train the same major muscle groups 2-3 times per day over the course of 6 or 7 days per week (Drechsler, 1998; Garhammer et al., 2003; Zatsiorsky, 1995). Furthermore, these athletes will endure sustained periods of intensified training (i.e. 2-3 wk of “overload”), followed by short-term periods (i.e. 1 wk) of “recovery”. The intensity, volume and frequency of high-power, HIRE that is performed by competitive weightlifters greatly exceeds the American College of Sports Medicine’s (ACSM) recommendations for improving muscular strength and power (Ratamess et al., 2009). The ACSM recommendations are supported by previous evidence demonstrating that repeated HIRE bouts of the same muscle groups can result in the persistent suppression of key anabolic mediators, prolonged inflammatory signalling and decrements in muscular performance (Coffey et al., 2007b; Fry et al., 1994b; 1994c; 2006b; Ratamess et al., 2003). However, in response to the cyclic pattern of overload and recovery weeks that are comprised of repeated daily HIRE sessions, weightlifters demonstrate both acute and long-term improvements in the performance of the competitive lifts and other related strength exercises (Crewther et al., 2010; 2011; Drechsler, 1998; Fry et al., 1994a; Häkkinen et al., 1988b; Pistilli et al., 2008; Stone et al., 1998). As these training methods are unmatched by other strength and power athletes, I proposed that highly-trained competitive weightlifters possess a unique ability to rapidly recover between repeated HIRE sessions within the same day, and following periods of overload. It has been proposed that the maintenance (or enhancement) of muscular function within and between successive bouts of HIRE may be attributable to acute changes in the architecture of skeletal muscle (Mahlfeld et al., 2004; Tillin et al., 2009). However,
such a structural mechanism is yet to be examined in competitive weightlifters. Further research in this area is of relevance to these athletes due to the large number of high-intensity muscle contractions that are performed by the same major muscle groups within each training session. Previous evidence has shown that it may take 2-5 weeks for the restoration and/or an increase in performance to occur in other athletes (e.g. swimmers, cyclists, American football players) following the cessation of overload periods ranging from 1-3 wk in duration (Fry et al., 1997; Halson et al., 2004; Moore et al., 2007; Stone et al., 1998). In contrast, competitive weightlifters exhibit a rapid restoration and/or improvement in performance following short-term periods of recovery training (i.e. 1 wk) (Crewther et al., 2010; Fry et al., 1993). As it may take 3-6 wks for peripheral adaptations to arise (i.e. increases in skeletal muscle cross-sectional area) (Phillips, 2000; Seynnes et al., 2007; Staron et al., 1994), it is evident that rapid systemic mechanisms enable weightlifters to positively adapt following shorter recovery intervals (i.e. 1 wk).

Thus, the acute systemic responses that are associated with this ability to rapidly restore neuromuscular function and competitive performance need to be explored further.

**Research Problem**

At present, the majority of the physiological research in competitive weightlifters has examined the acute neuromuscular and/or hormonal responses following singular training sessions (Häkkinen et al., 1988a; Kraemer et al., 1992; Liu et al., 2005; McMillan et al., 1993; Passelergue et al., 1995). Although such research is highly valuable, there is still much to be learnt about how these athletes respond and adapt to their uniquely intensive training structures that involve multiple daily training sessions and repeated overload and recovery periods.

To help explain the adaptive responses to resistance exercise, researchers are investigating the transcriptional and translational mechanisms that contribute to overall changes in systemic physiology (Carlson et al., 2011; Mahoney et al., 2008; Roth et al., 2002). Such research is highly informative as chronic adaptations that occur in skeletal muscle are likely to arise due to the summation of many single (acute) bouts of exercise which leads to a cumulative, global alteration in gene and protein expression (Pilegaard et al., 2000; Stepto et al., 2009). However, to date, the majority of such research has only examined the acute
responses in untrained and/or recreationally trained individuals following exercise protocols of moderate intensity and low to moderate volume (Dennis et al., 2004; Drummond et al., 2008b; Friedmann-Bette et al., 2011; Gordon et al., 2011). As an individual’s training status has been shown to influence the gene expression response to exercise (Brutsaert et al., 2006; Coffey et al., 2006a; 2006b; Nedergaard et al., 2007; Stepto et al., 2009), the results obtained from such investigations cannot necessarily be generalized to highly-trained athletes. Very few investigations have attempted to examine the acute molecular and/or neuromuscular responses in well-trained adults following high-power, HIRE protocols that are common to competitive weightlifters and other strength and power athletes (Lamas et al., 2010; Lehti et al., 2009; Stepto et al., 2009).

The early recovery phase from resistance exercise is characterized by acute inflammatory processes that promote skeletal muscle repair and regeneration (Clarkson et al., 2002; Peake et al., 2005a; Smith et al., 2008). As nutrient availability pre- and post-exercise serves as a potent modulator of many acute responses and chronic adaptations to resistance exercise (Braun et al., 2004; Hargreaves et al., 2002; Hawley et al., 2006; 2007; 2010; Roberts et al., 2010), there is a need to examine both systemic and local (i.e. in skeletal muscle) inflammatory responses following high-power, HIRE and feeding. Such research will enable a greater understanding of how post-exercise nutrition can influence various pathways involved in the recovery from HIRE in competitive weightlifters and other strength and power athletes.

In this thesis, a detailed examination of the acute molecular and neuromuscular responses to high-power, HIRE is conducted in competitive weightlifters and well-trained adults at the whole human level (i.e. performance, functional and systemic responses) and the specific tissue level (i.e. skeletal muscle and peripheral blood) using an integrative biological approach. The major goal of this thesis is to determine, at multiple levels, key physiological variables that contribute to the early recovery and adaptive processes following high-power, HIRE in competitive weightlifters and resistance-trained adults (refer to Figure 1-1).
Figure 1-1: Basic overview of the physiological responses examined in this thesis.
Thesis Overview

In Chapter 2, a review of the literature relevant to all major aspects of this thesis is presented. Firstly, the unique aspects of competitive weightlifting performance, training and physiology are identified and discussed to provide a context as to why the exercise-induced responses of competitive weightlifters may differ from resistance-trained and non-trained adults. Next, the structural, functional and oxidative stress responses and adaptations to frequent HIRE are presented to enable comparisons to be made between resistance-trained and/or non-trained adults and competitive weightlifters. In addition, the concept of resistance exercise overreaching and overtraining is discussed with particular focus on the influence of training volume and intensity on the development of these syndromes. The gene expression responses to resistance exercise are then summarized in addition to the influence of pre- and post-exercise feeding on these processes. Although no literature currently exists on the genomic responses of competitive weightlifters, the literature that is presented will address the results that have relevance to this thesis (i.e. peripheral blood and skeletal muscle gene expression in responses to exercise). Finally, the chemokine functional responses to resistance exercise are summarized with particular focus on Stromal-Cell Derived Factor-1 (SDF-1), Macrophage inflammatory protein-1β (MIP-1β), Monocyte Chemotactic Protein-1 (MCP-1) and C-X-C Chemokine Receptor Type 4 (CXCR4), due to the relevance of these genes in Study 1 and Study 3.

In Chapter 3, the specific aims and objectives of the three experimental studies, included in this thesis, are presented.

The experimental work is presented in Chapters 4, 5 and 6. To identify and examine novel systemic responses associated with the recovery from and adaptation to variations in training load in competitive weightlifters, Chapter 4 documents the gene expression and protein oxidation responses, alongside changes in performance and psychological indices, following 2 wk of overload and 1 wk of recovery (Study 1). Furthermore, to test the hypothesis that competitive weightlifters posses a greater ability to rapidly recover from successive HIRE bouts that are performed on the same day, the skeletal muscle structural and functional responses to “double-day training” are compared between competitive weightlifters and resistance-trained adults (Chapter 5 - Study 2). Finally, to extend the findings of Study 1, the acute effects of post-exercise feeding on the systemic and local inflammatory cytokine
responses to high-power, HIRE, similar to that performed by competitive weightlifters, is examined (Chapter 6 - Study 3).

There are several novel aspects in the design and outcomes of each of these studies. For Study 1, these included the recruitment of International-level competitive weightlifters, the use of microarray technology to identify exercise-responsive genes during a real-life training period in these athletes, the quantification of protein oxidative responses and the assessment of psychological indices during periods of overload and recovery training in weightlifters. In Study 2, a reliable, valid and non-technique dependent test to quantify neuromuscular function in weightlifters and resistance-trained adults was developed. In addition, the acute skeletal muscle architectural responses following repeated HIRE were assessed. Finally, during Study 3, the dual assessment of gene expression changes in PBMCs and skeletal muscle were performed following high-power, HIRE, with and without feeding during recovery.

In closing, Chapter 7 and Chapter 8 discuss the collective works as a whole, with particular emphasis on the potential applications of the research findings and the possible directions for future research. Independently and collectively these studies contribute to the understanding of the molecular and neuromuscular responses and adaptations that occur during the recovery from HIRE in competitive weightlifters and resistance-trained adults.
2.0 REVIEW OF LITERATURE
2.1 Unique Aspects of Competitive Weightlifting: Performance, Training and Physiology

Sections 2.1.1 to 2.5.4.3 appear as published:


2.1.1 Introduction

Weightlifting has been a longstanding part of the modern Olympic Games and has wide and growing international participation. During the performance of the two competitive lifts, the snatch and the clean and jerk (C&J), weightlifters are required to generate extremely high peak forces and contractile rates of force development and, consequently, high peak power outputs and contractile impulses (Garhammer, 1980, 1982, 1985, 1991, 1993; Storey et al., 2012).

This review details the unique performance and training requirements of competitive weightlifters with particular emphasis on the movement demands, training intensities and commonly adopted nutritional practices of these athletes. Further attention is directed towards descriptions of the physiological responses and adaptations of the musculoskeletal, cardiovascular and endocrine systems to weightlifting training and competition. Finally, as weightlifting is becoming increasingly popular with females and younger and older individuals, we highlight potential areas for future research that will enable the development of safe and effective training guidelines for these populations.

2.1.2 Literature Reviewed

The search for scientific literature relevant to this review was conducted using the U.S. National Library of Medicine (PubMed), SPORTDiscus™ and Google Scholar databases. Key search terms of ‘Olympic weightlifting’, ‘weightlifter/s’, ‘snatch’, ‘clean and jerk’, ‘muscular strength’ and ‘muscular power’ were used. Further literature was obtained from electronic ‘related articles’ searches and by manually screening the reference lists of included studies.
The inclusion criteria for all articles were; (i) refereed articles published in English language journals and books from the 1970s until February, 2012; and (ii) the terms ‘weightlifter’ and ‘weightlifting’ had to be in context with the sport of competitive weightlifting as opposed to general weight/resistance training.

2.2 Weightlifting Performance

The snatch and C&J are complex whole-body movements encompassing a series of high-intensity muscular contractions. During these lifts, weightlifters achieve power outputs unmatched by any other athletes (Garhammer, 1980). Since 1998, the recognized body weight classes are: men: $\leq 56\text{kg}$, $\leq 62\text{kg}$, $\leq 69\text{kg}$, $\leq 77\text{kg}$, $\leq 85\text{kg}$, $\leq 94\text{kg}$, $\leq 105\text{kg}$ and $>105\text{kg}$; and women: $\leq 48\text{kg}$, $\leq 53\text{kg}$, $\leq 58\text{kg}$, $\leq 63\text{kg}$, $\leq 69\text{kg}$, $\leq 75\text{kg}$ and $>75\text{kg}$. Athletes must weigh-in during a 1-hour window that begins 2 hours before the start of their competition session. The athlete’s placing within their respective body weight class is determined by their competition total which is the sum of their highest recorded snatch and C&J.

2.2.1 The Snatch

The snatch requires the weighted barbell to be lifted from the floor (using a wide grip) to an overhead position in one continuous movement (Garhammer, 1989). The snatch includes six phases (Figure 2-1). The first pull is initiated when the lifter extends their knees to raise the barbell off the platform to a position just below knee-level. A transition period (also referred to as the ‘double-knee bend’) follows whereby the knees are re-bent and are moved under the barbell whilst the lifter’s trunk is moved to a near vertical position (Enoka, 1979; Enoka, 1988; Stone et al., 2006b). The ‘double-knee bend’ allows the lifter to take advantage of a stretch-shortening cycle during the subsequent second pull (Stone et al., 2006b). The second pull requires the lifter to maximally accelerate the barbell by simultaneously shrugging the shoulders and extending the hips, knees and ankles. During the performance of near maximal to maximal full snatch attempts, the vertical velocity of the barbell during the second pull can range between 1.65 m/sec and 2.28 m/sec (Akkus, 2011; Campos et al., 2006; Chiu et al., 2010; Garhammer, 1991; Gourgoulis et al., 2000; 2002; Hoover et al., 2006). During sub-maximal attempts and snatch related movements (i.e. power snatch), barbell velocities may exceed 3.00 m/sec (Häkkinen et al., 1984c; Winchester et al., 2009). As the barbell rises in the vertical plane to ~62% - 78% of the lifter’s height (Akkus, 2011; Chiu et al., 2010;
Gourgoulis et al., 2000; 2002), the lifter begins to ‘pull’ their body underneath the barbell; this phase is referred to as the turnover. The lifter then ‘catches’ the barbell in a straight-arm overhead position whilst flexing at the knee and hip into a full squat position. The lifter then ‘recovers’ out of the full squat to a standing position whilst maintaining the barbell overhead. The duration of effort from the start of the first pull until the competition referees signal a successful lift is ~3-5 seconds. Each athlete is entitled to three snatch attempts in competition.

**Figure 2-1:** The six phases of the snatch: (a) first pull; (b) transition to the start of the second pull; (c) completion of the second pull; (d) turnover; (e) catch; (f) recovery.
2.2.2 The Clean and Jerk

The C&J is a two-part lift that enables heavier loads (~18-20\% greater) to be lifted than during the snatch. The clean requires the barbell to be raised from the floor (using a shoulder width grip) to the front of the shoulders in one continuous movement. There are six phases of the clean (Figure 2-2). The mechanical principles behind the first three phases (first pull, transition/double-knee bend and second pull) are the same as those of the snatch. During the second pull of near maximal to maximal attempt cleans, the vertical velocity of the barbell can range from 0.88 m/sec to 1.73 m/sec (Garhammer, 1985, 1991). However, during submaximal attempts and clean-related movements (i.e. power clean), barbell velocities may exceed 2.50 m/sec (Cormie et al., 2007; Winchester et al., 2005). As the barbell rises in the vertical plane to ~55\% - 65\% of the lifter’s height (Drechsler, 1998), the lifter initiates the ‘turnover’ phase. The lifter then ‘catches’ the barbell on their shoulders and descends into a full squat position. The lifter then ‘recovers’ from the full squat position to prepare for the jerk.

The jerk also has six phases (Figure 2-2): (i) start; (ii) dip; (iii) jerk drive; (iv) unsupported split under the bar; (v) supported split under the bar; and (vi) recovery. During the start phase, the lifter and the barbell must become motionless. The lifter then begins to dip down by flexing at the knee and hip, with the barbell held across the shoulders. At the lowest point of the dip, the lifter makes the transition to the jerk drive where they are required to accelerate the barbell in the vertical plane. During this transition period, the athlete may be exposed to a downward force equivalent to 17 times their body mass (Zernicke et al., 1977). Reported power outputs during maximal attempt jerk drives range from 2,140 watts (W) for a lifter in men’s under 56 kg class to 4,786 W for a lifter in the men’s 105kg+ class (Garhammer, 1980). At the completion of the jerk drive, the barbell is vertically driven off the shoulders and the lifter’s feet leave the ground. This phase represents the ‘unsupported split under the bar’. Once the lifter’s feet are in contact with the ground and the barbell is held overhead with fully extended arms, the lifter is in the ‘supported split under the bar’ phase. The lifter must then recover and is required to stand motionless with their feet parallel to one another. The duration of effort from the start of the first pull to the signal of a successful lift is ~8-12 seconds. Each athlete is entitled to three C&J attempts in competition.
Figure 2-2: The twelve phases of the clean and jerk: A) first pull; B) transition to the start of the second pull; C) completion of the second pull; D) turnover; E) catch; F) recovery from the clean; G) start position for the jerk; H) jerk dip; I) jerk drive; J) unsupported split under the bar; K) supported split under the bar; L) recovery from the jerk.
2.3 Training

There is limited evidence comparing the performance and physiological responses arising from different weightlifting training programmes (González-Badillo et al., 2006; Hoffman et al., 2004; Poletaev et al., 1995; Stone et al., 2006a). However, the English language coaching literature and empirical evidence suggests that numerous and varied practices exist amongst Internationally competitive weightlifters (Drechsler, 1998; Garhammer et al., 2003; Poletaev et al., 1995; Stone et al., 2006a; Thrush, 1995).

2.3.1 Exercises

The two competitive lifts form the basis of the training programmes for junior and senior weightlifters. Complementary exercises that have movement patterns similar to the competitive lifts (e.g. hang/power snatch, hang/power clean, snatch and clean pulls, front and back squats) and supplementary exercises (e.g. overhead presses, back extensions and abdominal work) that target synergistic muscle groups are also used. The complementary exercises are also incorporated into the training programmes of other power athletes (Ebben et al., 2001; Hoffman et al., 2004; Kilduff et al., 2007; Simenz et al., 2005) as follows: (i) kinematic similarities exist between the propulsive phases in both weightlifting and jumping movements; (Canavan et al., 1996; Carlock et al., 2004; Cormie et al., 2011a, 2011b; Garhammer et al., 1992; Hori et al., 2008) and (ii) significant relationships exist between weightlifting ability and power output during jumping ($r = 0.59$ to $0.93$) and sprinting ($r = 0.52$ to $0.76$) (Baker et al., 1999; Carlock et al., 2004; Channell et al., 2008; Hori et al., 2008; Tricoli et al., 2005) and tests of agility ($0.41$) (Hori et al., 2008).

However, despite commonalities in the mode of exercise and other acute variables, the training programmes of weightlifters differ, particularly in the frequency and volume of high-intensity loads, from that of other power athletes (refer section 2.3.3). The collective differences in the competitive demands and the required physiological adaptations of other various athletes may account for these discrepancies. Furthermore, due to the technically and physically demanding nature of the snatch and C&J, modified versions are often employed by other athletes for the enhancement of muscular power.
2.3.2 Annual Training Structure

Broad descriptions of variations in weightlifting training variables have been offered in the literature (Drechsler, 1998; González-Badillo et al., 2006; Hoffman et al., 2004; Pistilli et al., 2008; Poletaev et al., 1995; Stone et al., 2006a; Thrush, 1995). More specific details are rarely outlined. Due to the success of many Eastern European teams, in particular the former Soviet Union and Bulgaria, a number of the world’s training programmes are variations of the generalized training models established by these nations (Garhammer et al., 2003). However, Western coaches were required to make modifications to these training methods presumably due to the higher prevalence of anabolic steroid use amongst Eastern Bloc teams (Fair, 1988; Franke et al., 1997; Stone et al., 2006b); since the 1970s, competitive weightlifters have been subjected to random drug testing (Fair, 1988) that can include both urine and blood assays.

The training programmes from the former Soviet Union were based upon the classic ‘periodization’ model (Poletaev et al., 1995) consisting of a preparatory phase (generalized and specific conditioning), competition phase (specific training mimicking the demands of competition), and a transition phase (generalized conditioning at the end of a training cycle). A wide variety of exercises at varying intensities and volumes were incorporated into these programmes with the belief that this would prevent athletes reaching a state of overtraining due to ‘movement pattern monotony’ (Garhammer et al., 2003). Although International-level weightlifters would typically perform 20,000 – 25,000 multijoint exercise repetitions per year, only 15-35% of those repetitions were competition lifts performed at 80-90% of their one repetition maximum (1RM) with an additional 4-7% being performed at ≥90% of 1RM (Drechsler, 1998; Poletaev et al., 1995; Zatsiorsky, 1992).

In contrast, the Bulgarian training approach is characterized by frequent, near-maximal to maximal-intensity loading (Drechsler, 1998; Garhammer et al., 2003; Takano, 1989; Zatsiorsky, 1992; 1995) and is more closely aligned with the demands of competition. It has been reported that Bulgarian lifters performed between 1,400 and 4,000 maximal attempts and 450 and 460 failed supra-maximal attempts each year in training (Drechsler, 1998; Zatsiorsky, 1992). Approximately 10% of the total training time is devoted to warm up exercises, 45% to competition lifts, 40% to complementary strength exercises, 3% to supplementary exercises and 2% to other sports and cross-training activities (Drechsler,
There is very little variation in training intensity, by comparison with the training programmes of the former Soviet Union. However, fluctuations in training volume are applied. The training follows a repeated pattern of 2-3 weeks of increased loading followed by 1 week of reduced loading. This cyclic pattern of ‘overload’ and ‘recovery’ is believed to contribute to subsequent long-term improvements in performance (Pistilli et al., 2008; Wilson et al., 2008).

Although the competitive performances of weightlifters continue to improve, as evident by increases in National and World Records, further research needs to be directed towards several aspects of weightlifting program design. These aspects include: (i) effective coaching strategies for novice weightlifters; (ii) the influence of exercise volume and intensity on physiological and performance variables in female and youth weightlifters; and (iii) the efficacy of variations in training techniques (e.g. the incorporation of eccentric-only exercises).

### 2.3.3 Application and Variation in Training Load

International-level weightlifters perform two or more high-intensity resistance exercise (HIRE) [≥ 80% 1RM] sessions per day, of the same major muscle groups, 6 or 7 days per week (Drechsler, 1998; Garhammer et al., 2003; Poletaev et al., 1995; Stone et al., 2006a; Thrush, 1995). An extreme example of this high-frequency of training was demonstrated by the Greek weightlifting team during preparations for the 1996 Olympic Games. Across a 6-day training week, the Greek team performed 13 snatch, 11 C&J, 11 back squat and 9 front squat sessions (Drechsler, 1998). In senior weightlifters, dividing a given training volume across two sessions that are performed on the same day produces significantly greater increases in muscular strength, hypertrophy and maximal neural activation of the trained musculature (Häkkinen et al., 1994a; Hartman et al., 2007). However, as the majority of the exercises that are performed by weightlifters are the competitive lifts and similar multi-joint movements, a large number of muscle contractions are performed by the same major muscle groups within each training session.

Thus, the frequency of HIRE performed by weightlifters exceeds evidence-based recommendations for improving muscular strength and power in advanced trained adults. For example, the American College of Sports Medicine (ACSM) propose: (i) a training frequency
of 4-6 sessions per week; and (ii) training different muscle groups during subsequent strength and power sessions to allow for adequate recovery (Kraemer et al., 2004; Ratamess et al., 2009). Previous evidence has also shown that repeated HIRE bouts of the same muscle group/s result in the persistent suppression of key anabolic mediators, prolonged inflammatory signalling and decrements in muscular performance (Coffey et al., 2007b; Fry et al., 1994b; 1994c; 2006b; Ratamess et al., 2003). In contrast to these findings, weightlifters demonstrate both acute and long-term improvements in competitive lifting performance in response to their frequent HIRE training structure (Crewther et al., 2010; 2011; Drechsler, 1998; Fry et al., 1994a; Häkkinen et al., 1988b).

Although little evidence exists to suggest that weightlifting training, under proper supervision, is more injurious to children or adolescents when compared with other sports (Byrd et al., 2003; Faigenbaum et al., 1999; Hamill, 1994), considerable controversy still surrounds the use of weightlifting exercises in younger populations (i.e. <17 years of age). As such, definitive biological and/or training age appropriate weightlifting training guidelines have yet to be established. The training age of a weightlifter greatly influences their ability to positively adapt to the frequent use of HIRE. Over a 10-week training period in competitive Junior (17-20 years) weightlifters, moderate volumes of high-intensity (>90-100% 1RM) loading produced significantly greater strength gains (10.5% improvement in C&J and 9.5% improvement in back squat) when compared with low (3.0% improvement in C&J and 5.3% improvement in back squat) and high (6.9% improvement in back squat performance only) volumes of similarly high-intensity loading (González-Badillo et al., 2006). Furthermore, it has recently been demonstrated that performing additional high-intensity training sessions within the same day does not lead to significantly greater performance improvements in young weightlifters (Siahkouhian et al., 2010). In comparison, International-level senior weightlifters (20-35 years) demonstrate a greater ability to tolerate and adapt to higher volumes of high-intensity loading (Drechsler, 1998; Garhammer et al., 2003; Stone et al., 2006a; Storey et al., 2012). However, masters weightlifters (≥35 years of age) exhibit significant declines in training ability and weightlifting performance (Anton et al., 2004; Meltzer, 1994; Pearson et al., 2002; Thé et al., 2003) which is in accordance with the well documented impaired adaptive responses to resistance exercise with increasing adult age (Jozsi et al., 2000; Kumar et al., 2009; Newton et al., 2002). On the basis of these findings, we propose that an inverted U-shaped relationship exists between competitive age and the
volume of high-intensity loading that leads to enhanced weightlifting performance (Figure 2-3).

**Figure 2-3:** Proposed relationship between the volume of high-intensity (90-100% of one repetition maximum) loading that leads to enhanced weightlifting performance and competitive age. Junior: 17 to ≤20 years of age; senior: >20 to ≤35 years of age; masters: ≥35 years of age.

During ballistic activities such as bench throws or jump squats, absolute peak power output (PP) has been shown to occur between training loads of 30-50% of 1RM (Harris et al., 2000; Izquierdo et al., 2002; Kaneko et al., 1983; Newton et al., 1996; 1997; Stone et al., 2003b). However, more recent research suggests that the load required to elicit PP during jump squats may even be as low as body mass only (Cormie et al., 2007; Markovic et al., 2007; Nuzzo et al., 2010). Therefore, the prescription of relatively low-intensity (i.e. 0-60% 1RM) resistance exercise (inclusive of complementary weightlifting exercises) is often recommended to improve muscular power and dynamic athletic performance (Baker et al., 2001a, 2001b; Delecluse et al., 1995; Garhammer et al., 1980; Kaneko et al., 1983; McBride et al., 2002; Ratamess et al., 2009; Wilson et al., 1993). For example, the lighter relative training loads used for the power snatch, power clean and various pulling movements result in a greater maximum barbell vertical velocity, contractile impulse and thus a greater PP when compared with maximal competition lifts (Cormie et al., 2007; Garhammer et al., 1992; Kawamori et al., 2005; Thomas et al., 2007; Winchester et al., 2005; 2009). During the snatch and/or C&J, PP has been shown to occur with loads of 70-80% of 1RM (Cormie et al., 2007; Haff et al., 1997; Kawamori et al., 2005; Kilduff et al., 2007) demonstrating that the high-intensity
training of weightlifters results in improved PP under high load conditions. Therefore, weightlifters will frequently train for the competitive lifts at intensities \( \geq 70\% \) of 1RM (Drechsler, 1998; Garhammer et al., 2003; Poletaev et al., 1995; Stone et al., 2006a; Thrush, 1995). Athletes who are required to generate high PP against heavy external loads (e.g. wrestlers, bobsledders and rugby union/league players) are likely to benefit from high-load weightlifting training (Cormie et al., 2007; 2011b; Hoffman et al., 2004; 2009; Hori et al., 2005; 2008). However, at present there is a paucity of research examining the efficacy of power training with high- versus low-load weightlifting exercises in trained strength and power athletes.

### 2.3.4 Metabolic Cost of Weightlifting and Nutritional Practices

The metabolic demands of weightlifting training are reflected in the relatively high energy expenditures incurred by the athletes. For example, a mean caloric expenditure of 39.5 kJ/min was recorded in male weightlifters during a 1 week preparatory phase of training characterized by a moderate- to high-volume of moderate- to high-intensity lifts (Scala et al., 1987). This value is comparable with the metabolic cost incurred by high-volume circuit-style resistance exercise (Wilmore et al., 1978). Furthermore, the training stimulus alone produced a weekly energy expenditure of 16 456 kJ (Scala et al., 1987). The reported mean daily energy intakes of male weightlifters range between 13 212–19 307 kJ (Burke et al., 1988; 1991; Chen et al., 1989; Hassapidou, 2001; Marsit et al., 1998) which are consistent with the values recommended for ‘hard training’ male athletes (14 700 – 23 100 kJ/day) (Rogozkin, 2000). As expected, the corresponding relative daily energy intakes values of 134-244 kJ.kg\(^{-1}\).d\(^{-1}\) (Burke et al., 1991; Chen et al., 1989; Hassapidou, 2001; Marsit et al., 1998) are comparable with those of other strength and power athletes (Burke et al., 1991; Chen et al., 1989; Marsit et al., 1998; Sugiura et al., 1999). In regards to macronutrient consumption, it is reported that weightlifters consume a greater number of daily servings of protein-rich sources when compared with other athletes (Burke et al., 1988; 1991; Ronsen et al., 1999). As a result, the protein intake of male weightlifters has been reported to range between 1.6 - 3.2 g/kg/day (Chen et al., 1989; Hassapidou, 2001) which is high when compared with the recommended 1.2-1.7 g/kg/day for resistance training athletes (Burke et al., 1991; Chen et al., 1989; Häkkinen et al., 1988b; Maughan et al., 2002). Furthermore, weightlifters derive approximately 40-44% of their daily energy intake from dietary fat (Burke et al., 1991; Chen et al., 1989; Grandjean, 1989; Hassapidou, 2001), which is also
well above the acceptable range for health and athletic performance of 20-35% (Rodriguez et al., 2009b; Trumbo et al., 2002). This is a possible consequence of their greater intake of protein-rich animal products. Conversely, the reported carbohydrate intakes in weightlifters of 2.9-6.1 g/kg/day (Burke et al., 1991; Chen et al., 1989; Van Erp-Baart et al., 1989) are insufficient according to the current recommended levels of 7-8 g/kg/day for athletic individuals (Rodriguez et al., 2009a). Combined, these reports suggest that the dietary habits of male weightlifters may not yield the desired training gains and/or health benefits due to the emphasis placed on protein consumption (with high fat) at the expense of adequate carbohydrate ingestion. As the training and competition demands of weightlifters differ to those of other strength and power athletes, further research is required to: (i) document the current dietary habits of competitive weightlifters; and (ii) indentify the optimal macronutrient balance for weightlifting performance.

2.3.5 Influence of Body Weight Changes on Performance

Athletes participating in weight-restricted events will often train at a body mass that is 5-10% above their required competition weight class (Rogozin, 2000). In the week leading up to competition, a minor reduction in body mass (e.g. a loss of 1-2 kg) might be achieved by restricting fluid intake and consuming a low residue diet (Maughan et al., 2002). To ‘make weight’ and to avoid the loss of lean muscle mass, it is common for weightlifters to rapidly reduce total body water content prior to competition weigh in. This is achieved via passive methods including self-limited fluid intake, acute heat exposure and/or the use of (banned) diuretic agents (Judelson et al., 2007b). Whilst the detrimental effects of hypohydration on endurance performance are well documented (Cheuvront et al., 2005; Sawka, 1992; von Duvillard et al., 2004), less evidence exists regarding the effects of hypohydration on muscular strength and power. Evaluations of the effects of short-term hypohydration on maximal force production, muscular endurance and PP have demonstrated a decrease (Judelson et al., 2007a; Schoffstall et al., 2001; Torranin et al., 1979; Viitasalo et al., 1987; Webster et al., 1990) or no change (Fogelholm et al., 1993; Greiwe et al., 1998; Gutiérrez et al., 2003; Montain et al., 1998) in these variables. Where mild hypohydration (i.e. ≤2% reduction in body mass) techniques have attenuated neuromuscular performance, rapid rehydration interventions over a short period of time (i.e. over a 2 hour period as done in competition) have effectively restored performance variables (Schoffstall et al., 2001). However, an athlete’s ability to overcome the detrimental effects of dehydration is severely
affected when hypohydration-induced reductions in body mass reach 3-4% (Judelson et al., 2007b; Torranin et al., 1979).

It is thus tenable that severe hypohydration would impair weightlifting performance. For weightlifters opting to train at a body mass ≥3% above their competition weight, minor dietary modifications should be introduced in the weeks leading up to competition to achieve a body mass of ≤2% above that desired/required for competition. Mild hypohydration techniques may then be implemented 24 hours prior to competition weigh-in, followed by effective rehydration strategies afterwards.

2.4 Anthropometric Characteristics of Weightlifters

The anthropometric characteristics of male weightlifters have been documented extensively (Carter et al., 1984; Fahey et al., 1975; Fry et al., 2006a; Katch et al., 1980; Orvanová, 1990; Stone et al., 2006b; Tittel et al., 1992). Light- to middle-weight, male weightlifters (i.e. ≤56kg to ≤85kg) are somatotyped as predominately ectomorphic or mesomorphic (Orvanová, 1990) with body fat percentages of 5-10% (Fry et al., 2006a; Katch et al., 1980; Stone et al., 2006b; Tittel et al., 1992). These compositional characteristics are comparable to weight restricted wrestlers and athletes competing in the sprinting and jumping events of athletics (Kanehisa et al., 1999; Stone et al., 2006b; Thorland et al., 1981). Conversely, weightlifters in the heavy to unlimited weight classes (i.e. ≤94kg to >105kg) tend to be more endomorphic mesomorphs (Orvanová, 1990) with corresponding body fat percentages of ≥17% (Fahey et al., 1975; Stone et al., 2006b; Tittel et al., 1992). These individuals possess similar body compositions to heavyweight wrestlers, powerlifters, discus, shot put and hammer throwing athletes (Faber et al., 1990; Keogh et al., 2007; Kidd et al., 1983; Stone et al., 2003a; 2006b; Thorland et al., 1981). Although the anthropometric data on female weightlifters is less comprehensive, the limited data suggest that the body fat percentages of female weightlifters may be double that of male weightlifters of a similar body mass (Haff et al., 2008; Stoessel et al., 1991; Stone et al., 2006b). However, elite male and female weightlifters exhibit a lower body fat percentage when compared with lower level competitors of a similar total body mass (Fry et al., 2006a; Stoessel et al., 1991). Thus, the resulting differences in lean body mass become a major contributing factor to the divergent neuromuscular responses seen between male and female and elite versus. non-elite weightlifters (refer section 2.5.1.3) (Ford et al.,
2000; Frontera et al., 1991; Fry et al., 2006a; Marchocka et al., 1984; Maughan et al., 1983, 1984; Miller et al., 1993; Schantz et al., 1983; Stoessel et al., 1991).

In comparison to other strength and power athletes of a similar body mass and composition, weightlifters have proportionally shorter arm span and tibial lengths, larger biacromial breadths and are shorter in height (Carter et al., 1984; Fry et al., 2006a; Marchocka et al., 1984; Tittel et al., 1992). Such anthropometric characteristics provide two mechanical advantages when lifting maximal loads: (i) the mechanical torque that is required to lift a given load is less due to shorter lengths of the resistance lever arms; and (ii) the amount of muscular work required to lift a given load is decreased via a reduction in the vertical distance that the barbell must be displaced (Keogh et al., 2007). Furthermore, the shorter body dimensions coincide with a greater mean skeletal muscle cross-sectional area which is advantageous to weightlifting performance (Ford et al., 2000).

2.5 Physiological Responses and Adaptations to Weightlifting

The complexity, intensity and brevity of weightlifting impose great challenges when attempting to obtain valid and meaningful physiological data from competitive weightlifters. Furthermore, it is onerous and/or inappropriate to apply similar exercise protocols in non-weightlifters due to the technically demanding nature of the specific movements. As such there is limited data on the acute neuromuscular, cardiovascular and endocrine responses that occur (especially in female weightlifters) during weightlifting training and competition. Furthermore, few studies have examined the adaptions of the neuromuscular and endocrine systems that arise from moderate (weeks-months) to long-term (months-years) periods of weightlifting-specific training. However, investigations into the physiological responses and adaptions of masters weightlifters do provide some insight into the long-term benefits of weightlifting training.
2.5.1 Skeletal Muscle Structure and Function

2.5.1.1 Fibre Type Composition

The force-velocity properties of a muscle are in part determined by the relative proportions of fast-twitch (type IIA and IIX; formerly identified as IIB) and slow-twitch (type I) muscle fibres (Cormie et al., 2011a; Thorstensson et al., 1976a). Strength and power athletes, including weightlifters, exhibit mean percentages of fast-twitch fibres in the vastus lateralis ranging from 53% to 65% (Clarkson et al., 1980; Fry et al., 2003; Gollnick et al., 1972; Häkkinen et al., 1987a; 1988b; Prince et al., 1976; Tesch et al., 1984; Tesch et al., 1989). Although similar percentages have been reported in untrained adults (Gollnick et al., 1972; Ingjer, 1979; Simoneau et al., 1989), the cross-sectional areas of type II fibres are considerably larger in weightlifters (Fry et al., 2003; Gollnick et al., 1972; Tesch et al., 1984; 1989). Such a structural difference is advantageous to force production as type II fibres possess a greater capacity to generate power per unit cross-sectional area when compared with type I fibres (Bottinelli et al., 1999; Malisoux et al., 2006; Thorstensson et al., 1976a; Widrick et al., 2002). Both the proportion of type IIA fibres and the relative myosin heavy chain IIA isoform content have been shown to be greater in weightlifters when compared with recreationally active adults (Fry et al., 2003). In addition, weightlifting performance is strongly correlated to type IIA percent content (r = 0.94) and type IIA percent fiber area (r = 0.83) (Fry et al., 2003). Thus, evidence from cross-sectional studies suggests that the frequent high-intensity training of weightlifters results in hypertrophy of type IIA fibers (Fry et al., 2003; Gollnick et al., 1972; Tesch et al., 1984; 1989). Evidence from longitudinal HIRE studies in non-weightlifters indicates that there may also be a concomitant IIX to IIA fiber-type transformation (Campos et al., 2002; Fry, 2004; Green et al., 1999; Staron et al., 1990; 1994). Conversely, a restoration of type IIX content has been shown to occur in other athletes (i.e. swimmers, runners and cyclists) during pre-competition tapers which involve a planned reduction in training volume (Harber et al., 2004; Neary et al., 2003; Ross et al., 2001; Trappe et al., 2001; 2006). However, the existence of a tapering-induced re-shift in the fibre-type composition of competitive weightlifters has yet to be quantified.
2.5.1.2 Neuromuscular Function

Maximal voluntary isometric peak force (PF) and PP are strongly related to weightlifting performance (Haff et al., 2005; Hakkinen et al., 1986b; Kauhanen et al., 2000; Schmidtbleicher, 1992; Stone et al., 2005). During isometric conditions, PF is reached in the vicinity of 300 – 400 msec (Aagaard et al., 2002; Haff et al., 2005; Thorstensson et al., 1976b; Zatsiorsky, 2003). However, during dynamic weightlifting movements, weightlifters achieve PF, PP and maximum barbell velocities in <260 msec (Campos et al., 2006; Garhammer, 1991; Gourgoulis et al., 2000; 2009). Thus, the maximal contractile rate of force development (RFD) in the early phase of muscle contraction is of great importance to these athletes (Blazevich et al., 2008; Haff et al., 2005; Stone et al., 2004).

Improvements in both PF and contractile RFD have been reported in male and female weightlifters following moderate to long-term periods of training (González-Badillo et al., 2006; Haff et al., 2008; Häkkinen et al., 1987a; 1988b). These findings demonstrate that the frequent high-intensity training used by weightlifters (refer section 2.3.2 and 2.3.3) effectively increases muscular strength and power concurrently (Hakkinen et al., 1986b; Moss et al., 1997). As a result, the isometric PF and peak RFD of male weightlifters is ~15-20% and ~13-16% greater, respectively, when compared with other strength and power athletes (i.e. football players, sprinters, throwers and jumpers) (Häkkinen et al., 1988a; McGuigan et al., 2008; Nuzzo et al., 2008; Stone et al., 2008). This improved muscular function may arise due to an enhanced voluntary and/or reflex-induced neural activation of motor units (Aagaard et al., 2002; Häkkinen et al., 1988b; 1994a; Hartman et al., 2007) and/or a selective recruitment of fast-twitch motor units (Ewing et al., 1990; Nardone et al., 1989).

During the performance of the snatch and C&J, weightlifters have demonstrated some of the highest absolute and relative PP reported in the literature (Garhammer, 1980, 1982, 1991, 1993). For example, during the second pull of maximal snatch and C&J attempts, values as high as 5,442 W and 6,981 W, respectively, have been reported in male weightlifters (Garhammer, 1985, 1993). Furthermore, the corresponding relative PP for male and female weightlifters range from 53-56 W/kg and 38-40 W/kg, respectively, (Garhammer, 1991, 1993). As a comparison, during maximal bench press and deadlift exercises performed by male strength athletes, absolute PP of 415W and 1,274W, respectively, have been reported.
Garhammer, 1991, 1993) with relative PP ranging from ~4-12W.kg\(^{-1}\) (Garhammer, 1980, 1993). In addition, during exercise tests that incorporate the lower body (i.e. clean pulls and various jumps) the reported PP of male weightlifters is ~13-36% greater when compared with other power athletes (McBride et al., 1999; Nuzzo et al., 2008; Stone et al., 2003a; 2008). However, during upper body only exercise, no differences in absolute or relative PP were shown to exist between weightlifters and handball players (Izquierdo et al., 2002). These findings highlight the important contribution that the lower body makes to power development in weightlifters. Furthermore, they may be explained by the specificity of training, as handball players are required to perform repeated high-intensity upper body movements (i.e. throwing) in competition (Hoff et al., 1995; Izquierdo et al., 2002). Conversely, the upper body musculature of a weightlifter plays a relatively lesser role, in comparison to the legs, during the snatch and C&J (Bai et al., 2008; Izquierdo et al., 2002).

2.5.1.3 Sex- and Age-Related Differences in Neuromuscular Function

To compare performances across the different body weight classes, Sinclair scores, based upon current World record totals and adjusted each Olympic year, are used (Sinclair, 1985). The lifter’s actual competition total is multiplied by the appropriate Sinclair coefficient. The resulting score is a projection of the total the lifter would theoretically achieve if they were in the super heavy-weight class with the same lifting ability. Various other allometric scaling formulae have been derived (Batterham et al., 1997; Ford et al., 2000; Kauhanen et al., 2002; Siff, 1988). However, many tend to yield either an overestimation or underestimation for certain body weights.

In untrained and/or recreationally trained males and females, reported sex-related differences in absolute neuromuscular strength and power range from 31% to 48% and 17% to 46%, respectively (Kanehisa et al., 1994; Komi et al., 1978; Mayhew et al., 1990; Miller et al., 1993; Petrella et al., 2005). However, when comparing the current under 69kg (the only common body weight class between sexes) World record lifts for youth, junior and senior male and female weightlifters, there is a consistent sex-related difference of 15-20% (Table 2-1). Thus, it is evident that although long-term weightlifting training minimizes the sex-related difference in neuromuscular function, factors such as the distribution and total amount of lean body mass in male and female weightlifters will ultimately influence the expression of strength and power across all age and weight categories (Ford et al., 2000; Thé et al., 2003).
As competitive weightlifting is becoming increasingly popular in masters athletes, a number of studies have investigated the influence of increasing age on competitive weightlifting performance and neuromuscular function (Anton et al., 2004; Baker et al., 2010; Meltzer, 1994; 1996; Pearson et al., 2002; Thé et al., 2003). Pearson et al. (2002) demonstrated that, on average, masters weightlifters (aged 40-87) were able to generate 32% more isometric knee extensor force and lower body explosive power when compared with age-matched, healthy, untrained adults. It is likely that neural factors contributed to this enhanced functionality in older weightlifters as no significant differences in lean lower-limb volume existed between groups (Pearson et al., 2002). Furthermore, the maximal motor unit discharge rates in the rectus femoris of Masters weightlifters have been shown to be ~20% greater than in untrained, age-matched adults (Leong et al., 1999). Therefore, it appears that long-term weightlifting training and competition has the potential to attenuate the age-related decline in motor unit size, number and/or function that becomes apparent after the age of 60 years (Brown et al., 1988; Luff, 1998).

A significant reduction in type II fibre size and content is associated with increasing age (Larsson et al., 1978; Lexell et al., 1983; 1995) and is likely to contribute to the annual decline of ~1-1.5% in PP and competitive performance that has been reported in masters weightlifters (Meltzer, 1994; Pearson et al., 2002). According to Anton et al. (2004), the rate of decline in competitive performance is markedly greater in women across all weight classes (Anton et al., 2004). These previous findings are confirmed when comparing the current under 69kg male and female World Records across all age categories (Figure 2-4).
Interestingly, no such sex-related difference with increasing age has been reported during the expression of maximal strength in competitive powerlifting (Anton et al., 2004). Therefore, it was concluded that only the ability to perform complex and explosive power-type exercises declines at a greater rate in women (Anton et al., 2004). These findings are in accordance with previous investigations which have demonstrated that women undergo greater age-related declines in muscle shortening velocity and PP than men, which is likely due to a decreased neural drive and a combination of muscle fibre loss and atrophy (Krivickas et al., 2001; Trappe et al., 2003).

**Figure 2-4:** The sex-related differences as a function of age (years) between male and female World record totals in the under 69kg category. World record totals as of November, 2011. M = masters; $R^2$: coefficient of determination.
2.5.2 Bone Mineral Density

The skeletal structures of weightlifters undergo significant adaptations in response to the large compressive and shear forces that are encountered during training and competition (Calhoon et al., 1999; Garhammer et al., 1992; 1993; Zernicke et al., 1977). Biochemical indicators of bone formation are elevated by up to 35% in actively competitive weightlifters when compared with age-matched, healthy adults (Karlsson et al., 1995a). Furthermore, greater site-specific increases in trabecular and cortical bone densities have been reported in the vertebrae (13-42%), femoral neck/trochanter (12-24%), tibia (9-12%) and radius (10%) of competitive weightlifters when compared with untrained and recreationally trained adults (Conroy et al., 1993; Dinç et al., 1996; Heinonen et al., 2002; Karlsson et al., 1993; 1995b). Following ~30 years of retirement from weightlifting, former-weightlifters aged between 50 and 64 years have been shown to exhibit a significantly greater bone mass when compared with age-matched controls (Karlsson et al., 1995b). However, between the ages of 65-79 years, no differences in bone mass existed between groups (Karlsson et al., 1995b). Thus, it is evident that weightlifters must maintain an adequate level of physical activity past the age of 65 in order to attenuate age-related declines in bone mass.

2.5.3 Cardiovascular Structure and Function

High-intensity resistance exercise increases peripheral vascular resistance, thereby stimulating concentric left ventricular (LV) hypertrophy (Haykowsky et al., 2002; Richey et al., 1998). The increase in myocardial wall thickness arises due to the parallel addition of new myofibrils and is a compensatory attempt to reduce LV wall stress and systolic pressure (Grossman, 1980; Richey et al., 1998). A number of studies have examined the ventricular morphology and function in competitive weightlifters (Abinader et al., 1996; Adler et al., 2008; Brown et al., 1987; Fahey et al., 1975; Fleck, 1988; 1989; George et al., 1998a; 1998b; Gibbs, 1977; Lalande et al., 2007; Longhurst et al., 1980; Pearson et al., 1986; Pelliccia et al., 1993; Pluim et al., 2000; Snoeckx et al., 1982; Stone et al., 1983). Some investigations have reported that the absolute LV mass (g) of weightlifters may be ~13-30% larger than that of age-matched healthy and/or sedentary control subjects (Adler et al., 2008; Fleck et al., 1993; George et al., 1998b; Longhurst et al., 1980; Shapiro, 1984). However, in most instances, the increased LV mass exhibited by weightlifters is proportional to their total body mass, body surface area and/or lean body mass, thereby indicating a physiological as opposed to a
pathological adaptation (Fleck et al., 1993; George et al., 1998b; Longhurst et al., 1980; Shapiro, 1984). This is of importance as LV hypertrophy is categorized as an independent risk factor for cardiovascular morbid events (Richey et al., 1998). Conversely, other studies have shown no significant differences in absolute or relative measures of cardiac morphology between weightlifters and healthy adults (Brown et al., 1987; George et al., 1998a; Lalande et al., 2007; Pelliccia et al., 1993; Snoeckx et al., 1982). The lack of difference in these results may be explained by: (i) the experimental groups being more evenly matched for body dimensions (Brown et al., 1987; George et al., 1998a); (ii) differences in the training history of the control subjects (e.g. sedentary/untrained versus recreationally trained) (Snoeckx et al., 1982); and (iii) possible sex-related differences in cardiac hypertrophy as only one study has examined cardiac morphology in female weightlifters (George et al., 1998a). In light of these findings, the consensus of opinion is that weightlifting does not induce a true concentric enlargement of the left ventricle as seen in pathological conditions.

The cardio-respiratory function of competitive male weightlifters, as determined by maximal oxygen consumption ($VO_{2max}$), has been reported to range between 42.0 and 50.7 ml/kg/min (Fahey et al., 1975; Farrell et al., 1982; Fleck et al., 1993; MacFarlane et al., 1991; Snoeckx et al., 1982; Stone et al., 1983). As expected, these mean values are similar to those of other athletes involved in short-duration high-intensity/power activities (Fahey et al., 1975). Short-term (8 weeks) weightlifting-style training in active adults has been shown to increase both absolute and relative $VO_{2max}$ by ~6-7% (Stone et al., 1983). However, annual evaluations over the course of 3 years of specific weightlifting training in competitive weightlifters revealed significant reductions in both absolute and relative $VO_{2max}$ of ~4% and 11%, respectively (Nakao et al., 1995). With regards to resting haemodynamics, reported values for mean heart rates range between 60 beats per minute (bpm) and 81 bpm, systolic blood pressure between 115mmHg and 153 mmHg, and diastolic blood pressure between 71 mmHg and 93 mmHg (Adler et al., 2008; Fleck et al., 1993; Jost et al., 1989; Lalande et al., 2007; Longhurst et al., 1980; MacFarlane et al., 1991; Snoeckx et al., 1982; Stone et al., 1983). These data classify weightlifters as being ‘normal’ or Stage 1 hypertensive as per the ACSM guidelines (Armstrong et al., 2006).
2.5.4 Endocrine

Evidence of endocrine responses and adaptations in weightlifters and/or related to weightlifting performance are predominately limited to anabolic and catabolic hormones, for the large part in young male weightlifters. Here we include where substantial data have been obtained, the responses of testosterone, cortisol and growth hormone.

2.5.4.1 Testosterone

Testosterone is a potent androgenic-anabolic hormone that is considered to be a major promoter of muscular hypertrophy, strength and power (Cardinale et al., 2006; Vingren et al., 2010). The reported basal serum total testosterone concentrations in male weightlifters range from ~14.4-27.7 nmol/L (Blumert et al., 2007; Busso et al., 1992; Fry et al., 2000; Häkkinen et al., 1988a, 1988b; Izquierdo et al., 2004; Kraemer et al., 1992) which is within the normal range for young, healthy untrained men (12.1-34.7 nmol/L) (Izquierdo et al., 2004; Kraemer et al., 1998b; Tenover, 1992). Short-term exercise-induced increases in total serum testosterone of ~17-30% have been reported in male weightlifters in response to acute weightlifting training sessions of moderate to high-intensity and volume (Häkkinen et al., 1988a; Kraemer et al., 1992; Marsit et al., 1998). In addition to the influence of exercise type, volume and intensity, the training age of an individual also affects the exercise-induced testosterone response. Elite junior weightlifters with >2 years training experience exhibit significantly greater exercise-induced increases in serum testosterone when compared with those with ≤2 years training experience (Kraemer et al., 1992). Combined, these results demonstrate that weightlifting training elicits a response similar to that reported for conventional strength and hypertrophy protocols involving large muscle mass exercises (Ahtiainen et al., 2004; Häkkinen et al., 1993; Kraemer et al., 1990b; McCaulley et al., 2009).

The available data pertaining to the testosterone response to competition and competition-like settings is limited to salivary measures (Crewther et al., 2010; Passelergue et al., 1995). During official and simulated weightlifting competitions, Passelergue et al. (1995) reported no significant changes in salivary total testosterone. From this limited data, it appears that competition settings fail to meet the exercise volume threshold that is required to induce a significant testosterone response in weightlifters (Vingren et al., 2010). This is in accordance with previous investigations that have demonstrated no significant changes in total
testosterone following high-intensity, low-volume, moderately long rest period duration resistance exercise protocols (Bosco et al., 2000; Häkkinen et al., 1993; McCaulley et al., 2009; Smilios et al., 2003).

2.5.4.2 Testosterone : Cortisol Ratio

The basal testosterone : cortisol (T : C) ratio is often used to represent the physiological strain imposed by a training programme (Fry et al., 1997; Urhausen et al., 1995; 2002) as it generally exhibits an inverse relationship with exercise volume (Busso et al., 1992; Fry et al., 1993; Haff et al., 2008; Häkkinen et al., 1987b; Wu et al., 2008). For example, across a 5 week training period in elite female weightlifters, a 37.0% reduction in training volume elicited a mean increase of 72.5% in basal T : C ratios (Haff et al., 2008). Conversely, Wu et al. (2008) demonstrated that a 54% increase in weightlifting training volume over 2 weeks resulted in a 60% reduction in the basal T : C ratio. However, weightlifting training for ≥1 year and prior exposure to increased training volumes appears to attenuate this relationship (Fry et al., 1994a). Furthermore, during extended training periods (i.e. 12-24 weeks) of varying intensity and volume, experienced weightlifters have demonstrated a positive association between an increased basal T : C ratio and maximal voluntary isometric PF and PP (Haff et al., 2008; Häkkinen et al., 1985d; 1987b). Thus, the routine assessment of the basal T : C ratio may provide an effective way in which to measure acute and chronic adaptive responses to weightlifting training.

In competition settings, the T : C ratio is greatly influenced by pre-competition anxiety and may decrease prior to any form of strenuous physical activity (Passerergue et al., 1995). Furthermore, an official weightlifting competition has been shown to produce a higher salivary cortisol response, and thus a greater decrease in the salivary T : C ratio, when compared with a simulated competition (2011). However, competitors with higher pre-competition salivary cortisol levels also exhibited superior lifting performances (Crewther et al., 2011). In stressful situations, salivary cortisol levels have been shown to increase by 230% from basal values (Stahl et al., 1982). A pre-competition anticipatory rise in circulating catecholamines (French et al., 2007; Gibbs, 1977) may stimulate the release of adrenocorticotropic hormone, which in turn increases cortisol secretion (Al-Damluji, 1988). As a positive association exists between increased catecholamine levels and force production (French et al., 2007), it is possible that this mechanism may account for the higher cortisol
levels and the superior lifting performances that were reported during an official competition (Crewther et al., 2011).

2.5.4.3 Growth Hormone

Conventional resistance exercise does not appear to affect basal concentrations of growth hormone (GH) (Kraemer et al., 2005). In accordance with this contention, similar basal GH concentrations have been reported in male and female weightlifters (Fry et al., 1993; Häkkinen et al., 1988a; 1993; Kraemer et al., 1992; Stoessel et al., 1991) and other strength athletes (i.e. bodybuilders and powerlifters) when compared with recreationally trained and untrained adults (Ahtiainen et al., 2003; 2004; Kraemer et al., 1998b; 2005). Significant exercise-induced increases in GH occur similarly in men and women in response to moderate-intensity, high-volume and short rest period resistance exercise protocols (i.e. hypertrophy training) (Godfrey et al., 2003; Häkkinen et al., 1993; Kraemer et al., 2005). Conversely, only minor increases in GH have been reported following conventional strength and power protocols that use high-loads, low-repetitions and long rest periods (Goto et al., 2003; Häkkinen et al., 1993; Kraemer et al., 1990a; Linnamo et al., 2005). In contrast to these latter findings, 4.5-13-fold increases in GH have been reported in male weightlifters in response to their high-intensity, high-power training (Fry et al., 1993; Häkkinen et al., 1988a; Kraemer et al., 1992). However, these conflicting results may be explained by: (i) the differences in the training experience of participants that has been shown to affect the magnitude of GH release (Ahtiainen et al., 2003; 2004; Taylor et al., 2000); and (ii) differences in the absolute and relative intensity, volume and type of exercise performed (i.e. isolation versus multijoint exercise) in each investigation (Kraemer et al., 2005).
2.6 Physiological Responses and Adaptations to High Frequency, High-Intensity Resistance Exercise

Improvements in athletic performance will only occur if the intensity and volume of the exercise stimulus is above that of habitual activities; this forms the basis of the “overload principle” (Zatsiorsky, 1995). However, the fatigue and recovery responses to an exercise protocol are also critical factors which govern exercise-induced adaptation. In this section, the acute neuromuscular, skeletal muscle architecture and oxidative stress responses to HIRE will be discussed in conjunction with the concept of resistance exercise overreaching and overtraining.

2.6.1 Neuromuscular Responses

Peak force (PF) and contractile rate of force development (RFD) have strong relationships with athletic performance and, in some sports, the ability to generate force rapidly can be more important than maximal strength (Garhammer, 1993; McBride et al., 1999; Stone et al., 2003a). As weightlifters achieve maximum barbell velocities and peak power in <260 ms (Campos et al., 2006; Garhammer, 1991; Gourgoulis et al., 2000; 2009), the maximal contractile RFD, defined as the slope of the force-time curve (Figure 2-5) (Aagaard et al., 2002), is of great importance to these athletes (Haff et al., 2005). Furthermore, the contractile impulse (CI), defined as the integral of the force-time curve (Figure 2-5) (Aagaard et al., 2002), is another measure of muscular function relevant to weightlifting performance. Contractile impulse factors in the overall influence of the various time-related contractile RFD parameters (Aagaard et al., 2002; Baker et al., 1994). As mass remains constant during the performance of a weightlifting movement, an increase in CI would be associated with a higher movement velocity, a decrease in movement time and a greater displacement of the loaded barbell (Garhammer et al., 1992; Schilling et al., 2008). 

To enhance muscular function and competitive performance, weightlifters routinely perform successive HIRE sessions within the same day (as outlined in Section 2.3.3). Such a practice is not common to typical resistance exercise programs (Baechle et al., 2000; Ratamess et al., 2009) and PF has been shown to decline in resistance-trained adults when resistance exercise is performed twice during the same day (Chiu et al., 2004). Conversely, the RFD does not appear to be compromised in well-trained adults following two successive resistance exercise
sessions of differing intensity and modality (Chiu et al., 2004; Häkkinen et al., 1988a; 1992). To the best of our knowledge, no investigation has examined the acute CI responses to double-day training. Consistent with these previous findings, double-day programs are often split according to muscle group (e.g. upper vs. lower body) and training intensity (e.g. high vs. low) to enable recovery between subsequent sessions (Häkkinen et al., 1994a; Kraemer et al., 1998a). However, weightlifters are known to perform two or more HIRE sessions per day, inclusive of exercises for the same major muscle groups, six or seven days per week (Garhammer et al., 2003; Stone et al., 2006a; Zatsiorsky, 1995). It has been proposed that the maintenance (or enhancement) of muscular function within and between bouts of HIRE may be attributable to acute changes in the architecture of skeletal muscle (Mahlfeld et al., 2004; Tillin et al., 2009), as outlined in the following section.

**Figure 2-5:** An example force trace used to determine peak force, contractile rate of force development (RFD) and contractile impulse. Adapted and modified from Andersen et al. (2006).
2.6.2 Skeletal Muscle Architectural Responses and Adaptations

Skeletal muscle peak force, contractile RFD and CI are influenced by a number of morphological factors including muscle fiber type, tendon properties, pennation angle (\(\theta_p\)) and muscle thickness (Blazevich et al., 2007; Cormie et al., 2011a; Kawakami et al., 1995). Pennation angle (\(\theta_p\)), defined as the angle of the muscle fascicles relative to the points of insertion at the tendon or aponeurosis determines the arrangement of sarcomeres within a muscle (Aagaard et al., 2001; Narici, 1999). A large anatomical \(\theta_p\) allows more sarcomeres to be arranged in parallel. This in turn increases muscle thickness and physiological cross-sectional area (i.e. the cross-sectional area perpendicular to the line of fascicles) which are both positively associated with PF (Blazevich et al., 2007; Cormie et al., 2011a; Narici, 1999). In addition, muscle fibers of a greater \(\theta_p\) operate closer to their optimum length which is advantageous to force production (Blazevich, 2006). Conversely, smaller anatomical \(\theta_p\) allow more sarcomeres to be arranged in series which facilitates a rapid transmission of force to the tendon, thus increasing contractile RFD and CI (Fukunaga et al., 1997; Gans et al., 1991; Kawakami et al., 1993; Kumagai et al., 2000).

Acute changes in \(\theta_p\) occur in response to the recent history of previous muscular contractions. These changes appear to be influenced by the total exercise volume. High-power dynamic resistance exercise performed to failure and a high-volume of isometric maximal voluntary contractions (MVC) have been shown to acutely increase \(\theta_p\) by 10-11%, respectively (Csapo et al., 2011; Kubo et al., 2001). The acute increases in \(\theta_p\) following exhausting dynamic and repeated isometric contractions have been attributed to increased tendon compliance and muscle perfusion (Brancaccio et al., 2008; Csapo et al., 2011; Kubo et al., 2001). In contrast, an acute decrease in \(\theta_p\) of ~11% has been reported 3-6 min following 3 isometric MVC (Mahlfeld et al., 2004). Theoretically, an increased post-contraction \(\theta_p\), and consequently an increased PCSA, would increase PF. Conversely, a post-contraction reduction in \(\theta_p\) may provide a mechanical advantage for rapid force transmission to the tendon, thereby improving contractile RFD and CI. However, no investigation to date has determined the functional significance of such changes in skeletal muscle architecture.
2.6.3 Oxidative Stress Response

Free radicals are pro-oxidant molecules with one or more unpaired electrons in the valence shell (Cheeseman et al., 1993; Finaud et al., 2006; Jenkins, 1988). Reactive oxygen species (ROS) are a branch of free radicals (derived from oxygen) that are generated as a by-product of normal cellular metabolism, primarily in the mitochondria (Finaud et al., 2006; Thannickal et al., 2000). Small (physiological) amounts of ROS are a cellular requirement due to their involvement in signalling pathways which regulate a variety of cellular activities including cytokine secretion, growth, differentiation and gene expression (Finaud et al., 2006; Griending et al., 2000; Hensley et al., 2000; Sauer et al., 2001; Thannickal et al., 2000). However, in response to physiological stress such as exercise, ROS generation dramatically increases and has the potential to induce significant biological damage. (Alessio et al., 2000; Bloomer et al., 2004; Dalle-Donne et al., 2003b; Finaud et al., 2006; Goldfarb et al., 2005; Halliwell, 1996; Margonis et al., 2007a; Vollaard et al., 2005). To prevent such damaging effects, intracellular non-enzymatic (e.g. glutathione) and enzymatic (e.g. superoxide-dimutase, catalase and glutathione peroxidase) molecules and extracellular molecules (e.g. plasma uric acid, ascorbic acid, alpha-tocopheral and albumin) scavenge and buffer ROS (Ji et al., 2006; Turner et al., 2011). However, if pro-oxidant production overwhelms the body’s antioxidant defenses, a state of oxidative stress arises (Bloomer et al., 2005a; 2007; Dalle-Donne et al., 2003b).

Increasing ROS production exerts a detrimental effect on exercise performance and several possible mechanisms exist for this phenomenon. Oxidative damage to adenosine triphosphatase (ATPase) pumps can significantly reduce sarcoplasmic reticulum (SR) Ca\(^{2+}\) uptake (Scherer et al., 1986; Suzuki et al., 1991; Xu et al., 1997) which affects skeletal muscle excitation-contraction coupling. Furthermore, ROS-induced damage of ATPase pumps alters Na\(^+\)/K\(^+\) gradients thereby affecting the generation of subsequent action potentials (Sen et al., 1995). Finally, evidence suggests that skeletal muscle contractile proteins (MHC I, MHC II), and mitochondrial enzymes that are required for energy provision (succinate dehydrogenase, cytochrome oxidase) are susceptible to oxidative damage (Haycock et al., 1996).
Resistance exercise has been shown to increases ROS production in a biphasic manner. The first peak is caused by the repeated ischemia-reperfusion injury sustained during exercise and the second peak is dependent upon the accumulation of infiltrated phagocytic cells at the site of damage several hours post-exercise (Uchiyama et al., 2006). Although the invasion of phagocytic cells into the sites of muscle damage is essential for effective regeneration to occur, the ensuing magnitude of ROS generation can be such that previously undamaged muscle cells may sustain injury (Zerba et al., 1990). As oxidative stress increases proportionally to resistance exercise training volume, it is postulated that prolonged decrements in muscular strength (as seen in overtrained strength athletes: refer to Section 2.6.4) may be associated with ROS-induced muscle damage (Margonis et al., 2007a).

2.6.3.1 Measures of Oxidative Stress

The degree of oxidative stress can be estimated from the measurement of: 1) free radical content; 2) free-radical mediated damage on lipids, proteins or DNA molecules; and 3) antioxidant enzymatic activity or concentrations (Finaud et al., 2006). In general, reliable markers of oxidative stress must possess the following qualities: 1) be chemically unique and detectable, 2) increase or decrease accordingly during periods of oxidative stress, 3) possess relatively long half-lives, and 4) not be impacted by other cellular processes (e.g. cell cycle, energy metabolism etc) (Powers et al., 2008). When assessed in plasma, these biomarkers may provide a non-specific, whole body measurement of oxidative stress (Berlett et al., 1997; Dalle-Donne et al., 2003b; Finaud et al., 2006; Veskoukis et al., 2009). Commonly used measures of oxidative stress are outlined in Table 2-2.
Table 2-2: Commonly used methods to assess oxidative stress in biological systems.

<table>
<thead>
<tr>
<th>Lipid Peroxidation</th>
<th>Biological Sample</th>
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<tr>
<td>F2-isoprostanes</td>
<td>Plasma</td>
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<tr>
<td>thiobarbituric acid-reactive substances (TBARS)</td>
<td>Plasma</td>
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<tr>
<td>diene-conjugated compounds (DCC)</td>
<td>Plasma</td>
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<tr>
<td>Lipid hyperoxide</td>
<td>Plasma</td>
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<tr>
<td>Malondialdehyde (MDA)</td>
<td>Plasma</td>
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<tr>
<td>Oxidized LDL</td>
<td>Plasma</td>
</tr>
<tr>
<td>Pentane</td>
<td>Expired gas</td>
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<tr>
<td>Ethane</td>
<td>Expired gas</td>
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<tr>
<td>Hexane</td>
<td>Expired gas</td>
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<th>Protein Modification</th>
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<tr>
<td>Protein carbonyl</td>
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<td>Oxidized amino acids</td>
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<th>DNA Modification</th>
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<tr>
<td>nucleotide 8-hydroxy-2′-deoxyguanosine (8-OHdG)</td>
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<th>Antioxidant Measures</th>
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<td>Antioxidant vitamins (A, C and E)</td>
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2.6.3.2 Protein Carbonyls

Many different types of protein oxidative modifications can be induced either directly by ROS or indirectly by reactions of secondary by-products of oxidative stress (Berlett et al., 1997). Protein modifications elicited by a direct oxidative attack on lysine, arginine, proline or threonine or by secondary reaction of cysteine, histidine or lysine residues can lead to the formation of protein carbonyl derivatives (aldehydes and ketones) (Berlett et al., 1997; Dalle-Donne et al., 2003a). This form of protein modification has great relevance to human performance as carbonylated proteins are irreversibly damaged and lose their physiological function (Nikolaidis et al., 2008).
Increasing physiological stress can lead to the oxidation of blood and structural proteins resulting in an overall increase in the relative level of protein carbonyl (PC) groups (Chevion et al., 2000; Dalle-Donne et al., 2003b; Finaud et al., 2006). Protein carbonyl content is an early indicator of tissue damage and the formation of PC is also associated with a number of pathological conditions (Beal, 2002; Dalle-Donne et al., 2003a) ranging from Alzheimer’s disease (Aksenov et al., 2001; Choi et al., 2002; Conrad et al., 2000; Smith et al., 1991), to diabetes (Telci et al., 2000a; 2000b).

Protein carbonyls are suitable measures of training stress in athletes because their formation changes as a function of oxidative stress, they are stable in isolated body fluids and they do not show diurnal variation (Margonis et al., 2007b). Thus, PC levels in blood reflect the oxidative stress status in skeletal muscle (Veskoukis et al. 2009).

During resistance exercise, plasma and skeletal muscle PC concentrations may rise due to a number of reasons. Firstly, the disruption of iron containing proteins, such as erythrocytes, can lead to an increase in free iron content which is known to catalyze radical reactions (Halliwell et al., 1990; Stadtman et al., 1991). As all amino acids are susceptible to metal-catalyzed oxidation, an increase in ROS production consequently leads to an increased oxidation of amino acid side chains and fragmentation (Goldfarb et al., 2005; Stadtman et al., 1991). Secondly, a rise in PC concentrations after damaging resistance exercise (e.g. eccentric exercise) may be attributed to invasion of phagocytic cells at the site of damage several hours post-exercise (Bloomer et al., 2005a; Goldfarb et al., 2005; Uchiyama et al., 2006). Following a single bout of high-intensity eccentric exercise (60 eccentric contractions of the non-dominant arm elbow flexors with a load of ~135-150% of the dominant arm’s maximum isometric force), Lee et al. (2002) reported a significant increases of 83% and 62% in plasma PC concentrations 24 and 48 h post-exercise, respectively. Furthermore, DOMS scores were significantly elevated in the exercised arm 24-96 h post-exercise (the highest soreness value was recorded 48 h post-exercise) and were significantly correlated with the elevated PC concentrations (Lee et al., 2002). Bloomer et al. (2005b) also reported a similar association between DOMS scores and PC concentrations which were significantly elevated 6 and 24 h post-exercise in response to a moderate-intensity, high-volume resistance exercise (dumbbell squatting at 70% of 1RM for a 30 min period). Thus, it appears that measuring plasma PC content could serve as an effective tool for monitoring resistance exercise training stress in athletes.
2.6.4 Resistance Exercise Overreaching and Overtraining

The process of intensifying training for a short-term period (i.e. days to weeks) is commonly employed by athletes in an attempt to enhance performance (Halson et al., 2004; Zatsiorsky, 1995). However, in some instances, an excessive increase in training stress can result in negative physiological and performance responses. The term “overreaching”, is used to describe a planned accumulation of exercise training load (i.e. overload) that results in a short-term decrement in athletic performance (Halson et al., 2004). Following an appropriate reduction in training load (i.e. recovery), a restoration and/or improvement in performance (i.e. “supercompensation”) may occur within several days to several weeks (Baechle et al., 2000; Stone et al., 1999a, 1999b). Throughout the course of a training cycle, coaches intentionally expose their athletes to repeated overload and recovery periods to capitalize on the supercompensation phenomenon (Baechle et al., 2000; Stone et al., 1999a, 1999b). At present, very little is known about the exact physiological mechanisms that are associated with improved competitive performance following alterations in resistance exercise training load. However, if the planned recovery period is insufficient, the athlete may reach a state of “overtraining”, defined as a long term decrement in performance requiring several weeks to several months to resolve (Halson et al., 2004).

The concept of overreaching and overtraining coincides with the General Adaptation Syndrome (GAS) which displays three distinct stages (Selye, 1950, 1951). The first stage, termed the “General Alarm Reaction”, signifies the identification of a threat or stressor which initiates the fight-or-flight response by increasing sympathetic nervous system activity (Selye, 1950, 1951). The second stage, termed the “Stage of Resistance”, becomes evident if the stressor persists and the body attempts to adapt to the increased demands placed upon it (Selye, 1950, 1951). However, as the body is unable to maintain this heightened state indefinitely, the continued presence of the stressor will push the body into the third stage, termed the “Stage of Exhaustion” (Selye, 1950, 1951). At this point the body’s resources are depleted and the overall performance and functionality of the biological system can become severely compromised, as seen in cases of overtraining. Thus, an overreaching/overtraining continuum exists regarding human performance (Figure 2-6).
At present, the majority of the overreaching/overtraining literature is based on low-moderate intensity, high-volume aerobic-type exercise protocols. These studies have assessed a number of physiological factors including changes in heart rate (both at rest and during sub-maximal exercise) (Hedelin et al., 2000a; 2000b), mood states (Morgan et al., 1987), enzyme activities, metabolic markers in blood, hormone levels (Urhausen et al., 1995), immunological parameters (Rowbottom et al., 1995), and respiratory exchange ratios (Urhausen et al., 2002). However, as the nature of resistance exercise and the physiological adaptations that occur are distinctly different from that of aerobic-type exercise, common physiological responses between the two types of training cannot be assumed (Häkkinen et al., 1989a). Although much of the available scientific data on resistance exercise overtraining has been extrapolated from overreaching scenarios (Fry et al., 1997), a survey of overtrained athletes found that 77% were involved in sports requiring high levels of strength, speed and/or co-ordination. 

Figure 2-6: The overreaching and overtraining continuum which coincides with Hans Seyle’s General Adaptation Syndrome. Adapted and modified from Fry et al. (1997).
Thus, it is evident that strength and power athletes are highly susceptible to states of overreaching and/or overtraining and further research is required to investigate the physiological responses of these athletes to structured periods of overload and recovery.

2.6.4.1 The Role of Training Volume and Intensity

According to Fry & Kraemer (1997), the neuroendocrine responses to high-volume resistance exercise overreaching/overtraining are similar to those observed with aerobic exercise overreaching/overtraining. However, when resistance exercise intensity is increased substantially, a very different neuroendocrine response exists (Figure 2-7).

![Resistance Exercise Overreaching & Overtraining](image)

**Table:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Increased Training VOLUME</th>
<th>Increased Training INTENSITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>↓ Resting &amp; acute</td>
<td>↔ Or slight ↓ resting and acute</td>
</tr>
<tr>
<td>Cortisol</td>
<td>↑ Resting &amp; acute</td>
<td>↓ Resting &amp; acute</td>
</tr>
<tr>
<td>T:C Ratio</td>
<td>↓ Resting &amp; acute</td>
<td>↓ Resting &amp; acute</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>↔ Or slight ↓ resting and acute</td>
<td>↔</td>
</tr>
<tr>
<td>Creatine Kinase</td>
<td>Unknown</td>
<td>Normal training values</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Unknown</td>
<td>↑ Acute</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Unknown</td>
<td>↑ Acute</td>
</tr>
<tr>
<td>Lactate</td>
<td>↓ Acute</td>
<td>↓ Acute</td>
</tr>
</tbody>
</table>

*Figure 2-7:* Comparison of endocrine and blood-borne responses to resistance exercise overreaching and overtraining (rest = resting/basal; acute = in response to resistance exercise). Responses that are shaded in grey are also observed in response to aerobic exercise overreaching and overtraining. T:C ratio = Testosterone : Cortisol ratio. Adapted from Fry et al. (1997).
As previously mentioned in Section 2.5.4.2, the basal testosterone : cortisol (T : C) ratio is often used to represent the physiological strain imposed by a training stimulus (Fry et al., 1997; Urhausen et al., 1995; 2002). In response to a prolonged increase in training volume, a $\geq 30\%$ reduction in the T : C ratio (from a baseline measure) can occur. Such a response has been suggested as an indicator of a decreased level of ‘athletic preparedness’ (Haff et al., 2008). Following a 3 wk period of high-volume training in elite female weightlifters, 67% of the participants demonstrated a decreased level of athletic preparedness based upon the aforementioned criterion. In addition, six out of eight isometric and dynamic performance measures were negatively affected during the overload period (Haff et al., 2008). Conversely, no changes in weightlifting performance have been shown to occur in male weightlifters following shorter periods of increased training volume (i.e. 1-2 wk), despite significant reductions in their T : C ratios (Fry et al., 1993; 1994a; Häkkinen et al., 1987b). The different results obtained from these investigations may be explained by: (1) the length of the overload period; it appears that performance decrements in competitive weightlifters are associated with a specific training volume threshold (i.e. $>2$ wk of increased volume); (2) differences in the performance measures used (i.e. isometric and dynamic clean pull tests versus snatch and clean and jerk performance); and (3) the smaller reduction in the T : C ratios reported for male weightlifters which may have been a result of the shorter overload period or possibly due to sex-related differences.

The persistent fatigue and performance decrements that have been reported following high-volume training may be attributed to changes in metabolic responses (e.g. lower submaximal and maximal blood lactate concentrations), hormone levels (Figure 2-7), immune system status and substrate availability (e.g. the occurrence of a “metabolic end point” where muscle glycogen and plasma glucose levels are reduced and plasma free fatty acid levels are elevated) (Halson et al., 2004; Meeusen et al., 2006b; Stone et al., 1998; Urhausen et al., 2002). However, a complex combination of both peripheral and central factors is believed to contribute to the adverse signs and symptoms that result from significant increases in training intensity. For example, following a 2 wk HIRE protocol that was designed to induce overreaching (10 x 1 repetition at 100% 1 RM daily for 2 weeks), significant decrements in both voluntary and involuntary quadriceps force production occurred, indicating a maladaptation in the peripheral musculature and a decrease in central drive (Fry, 1998). Although such an intense protocol is not common to typical resistance exercise programs, some competitive weightlifters have adopted a similar training approach with varying degrees
of success (Drechsler, 1998; Fry, 1998; Stone et al., 1993; Zatsiorsky, 1992). At present, very little is known about the physiological responses to such intense loading structures in highly-trained competitive weightlifters who are able to tolerate much higher-intensity training programs when compared with resistance-trained adults (Kraemer et al., 1998c).

During the early stages of HIRE overreaching, it has been proposed that the body engages all potential repair processes (e.g. increased inflammatory cell recruitment) to offset the extreme physical demands along with increasing sympathetic nervous system activity at rest (“sympathetic syndrome”) (Fry et al., 1997; Kraemer et al., 1998c). Sympathetic syndrome is predominant in overreached individuals who train for speed and/or power (Fry et al., 1991; Stone et al., 1991) and is believed to occur in an attempt to maintain performance levels and physiological homeostasis (Halson et al., 2004). Several other studies have also suggested that contractile speed and muscular power may be attenuated more than muscular strength in response to HIRE overreaching (Fry et al., 2006b; Haff et al., 2008; Häkkinen, 1992). These findings are of particular relevance to competitive weightlifting due to the high-intensity, explosive nature of the sport. As certain kinematic parameters (e.g. maximum barbell acceleration, velocity and displacement) must be achieved in order to successfully perform weightlifting movements (Garhammer, 1985, 1991, 1993), further research is required to investigate possible mechanisms that may enable weightlifters to maintain (or enhance) rapid contractile function during intensive training periods comprised of repeated HIRE sessions.

### 2.7 Gene Expression Responses to Resistance Exercise

Acute bouts of resistance exercise produce time- and intensity-dependent patterns of gene expression that are easily detectable in isolated mononuclear cell fractions (Carlson et al., 2011) and skeletal muscle (Drummond et al., 2008b; Kostek et al., 2007; Liu et al., 2010; Pilegaard et al., 2000; Roth et al., 2002; Yang et al., 2005).

A number of factors influence the genomic responses to resistance exercise and these include the sex, age, training experience and nutritional status (e.g. exercising in a fed versus non-fed state and consuming or abstaining from a post-exercise meal) of an individual, the exercise modality and the type of muscle contraction/s performed, the time of day at which the biological sample was taken, and the use of ergogenic aids (e.g. creatine and/or amino acid.
supplementation) (Deldicque et al., 2008; Hulmi et al., 2009; Liu et al., 2010; Mahoney et al., 2008; Roth et al., 2002).

The majority of resistance-exercise induced gene expression literature has focused on the acute responses in untrained individuals following moderate-intensity, low- to moderate-volume exercise protocols (Dennis et al., 2004; Drummond et al., 2008b; Friedmann-Bette et al., 2011; Gordon et al., 2011). Very few investigations have examined the acute responses in well-trained individuals following high-power, HIRE protocols that are common to competitive weightlifters and many other strength and power athletes (Lamas et al., 2010; Lehti et al., 2009; Stepto et al., 2009).

In this section, the current evidence regarding the genomic responses to resistance exercise protocols of differing intensity, modality and duration will be discussed. Results obtained from high-throughput techniques (i.e. microarray) and stringent quantification methods (i.e. real-time quantitative PCR) performed in peripheral blood will be contrasted against those obtained from skeletal muscle. Particular emphasis will be placed upon a number of genes that are associated with inflammatory responses and mechanical signalling processes. Finally, as mRNA levels may not reflect the cellular protein levels of a specific transcript (Bustin, 2002), protein quantification data will be included where possible.

2.7.1 Gene Expression in Peripheral Blood Mononuclear Cells

Blood is classified as a fluid tissue which functions to connect the entire biological system (Liew et al., 2006). Monitoring changes in blood gene expression is becoming a popular diagnostic and research tool as it is the most accessible biological source to perform measurements of stress parameters on the cellular and molecular level (Fehrenbach, 2007).

Gene expression analyses of whole blood samples demonstrate a high variability due to the large abundance of neutrophils and reticulocytes (Debey et al., 2004). On this basis, neutrophils and reticulocytes are often excluded from genomic analyses as it is felt that their presence might obscure subtle changes in the more active lymphocyte and monocyte populations (Connolly et al., 2004b). Therefore, it is common practice to perform gene expression profiling on isolated lymphocytes and monocytes, which are collectively termed
Peripheral blood mononuclear cells (PBMCs). This method has proven to be an effective approach at reducing unwanted inter-sample variation when performing gene transcription profiling in blood (Eady et al., 2005; Radich et al., 2004).

Previously, it has been demonstrated that blood cells can mimic the gene expression-profile alterations that are known to occur in muscular adaptations (Zeibig et al., 2005). In addition, the less invasive nature of blood sampling (when compared to biopsy procedures) also allows repeated measures to be taken from the same participant, on the same day, with relative ease. For example, via the use of an indwelling catheter, Radom-Aizik and colleagues (2009a; 2009b) were able to obtain 40 blood samples (for the purposes of microarray analyses) from late pre-pubertal males and females during a constant-work rate cycle ergometry protocol. Therefore, assessing changes in peripheral blood gene expression is becoming an attractive option to those wishing to investigate the time course of adaptation to exercise.

BD® Vacutainer CPT™ Cell Preparation Tubes are a convenient and effective method with which to isolate PBMCs. The CPT™ blood collection tubes contain a sodium citrate anticoagulant with a FICOLL™ Hypaque™ density fluid and a thixotropic polyester gel barrier. Following routine blood collection methods and a series of centrifugation and wash steps, lymphocytes and monocytes can be isolated for genomic analyses. However, care must be taken when interpreting the results of gene expression studies whereby the tissue samples have been prepared in vitro. For example, the additional sample processing steps that are required to isolate PBMCs may incite a gene expression “signature” that is characteristic of prolonged handling of human cells and tissues (Eady et al., 2005; Rainen et al., 2002; Whitney et al., 2003). Therefore, this may prevent the direct comparison of genomic results obtained from whole blood versus those obtained from fractioned blood samples.

Furthermore, the time delay factor between the acquisition of a blood sample and the isolation of total RNA can greatly influence the resulting gene expression profile (Debey et al., 2004; Radich et al., 2004). To investigate this variable, Debey et al. (2004) compared the gene expression profiles from total RNA that was isolated under immediate (i.e. RNA was isolated within 15 min of blood sample acquisition) and delayed conditions (i.e. RNA isolation was delayed for 20-24 h). The delayed samples exhibited an up-regulation of genes associated with hypoxia and stress responses and a downregulation of genes associated with metabolism, cell cycle, apoptosis and immune function. It was concluded that these changes
were not due to degradation of RNA, but rather due to a cellular response to factors such as hypoxia and hypothermia (Debey et al., 2004). Based upon these findings, it is imperative that RNA isolation is performed as soon as possible following sample acquisition in order to limit any aberrant findings.

Microarray technology enables the simultaneous analysis of thousands of genes that are derived from various biological sources, including PBMCs (Fehrenbach et al., 2003). Exercise-based microarray studies provide insight into a number of signalling responses and molecular mechanisms that are critical to the early response to exercise and the late adaptation process.

To date, the vast majority of microarray studies that have documented the PBMC transcriptional responses to exercise have utilized aerobic-based protocols of varying intensities (i.e. 60-80% VO$_{2\text{max}}$) (Büttner et al., 2007; Connolly et al., 2004b; Moldoveanu et al., 2000; Radom-Aizik et al., 2009a; Zieker et al., 2005). The acute (i.e. 0 – 3 h post-exercise) transcriptional responses to such exercise has been well documented. However, very few investigations have examined the short-term genomic responses (i.e. 24 h+) following aerobic exercise (Moldoveanu et al., 2000; Zieker et al., 2005). Furthermore, to date, only one PBMC microarray investigation has been performed following a 30 min, moderate-intensity, moderate-volume resistance exercise protocol (six sets of the parallel back squat, followed by six sets of the seated leg press at ~65% of 1RM) in moderately-trained individuals (Carlson et al., 2011). Using a conservative two-fold change or higher threshold criteria, Carlson et al. (2011) demonstrated that the greatest gene response occurred 2 h post-resistance exercise in pathways related to immune response, inflammation and cellular communication (Carlson et al., 2011).

In comparison, following 30 min of intense aerobic exercise (performed at ~80% of VO$_{2\text{max}}$), a predominately pro-inflammatory response has been reported immediately post-exercise with the up-regulation of inflammatory cytokine genes such as MIP-1α, MIP-1β and IL18RAP, and stress proteins such as HSPH1 and heat shock 70-kDa protein 1A (HSPA1A) (Büttner et al., 2007; Connolly et al., 2004a; 2004b).

Combined, these results demonstrate that quantification of exercise-induced gene expression changes in peripheral blood is a viable and possibly more accessible research option. The less
invasive nature of this method has the potential to further our understanding of the acute molecular responses to resistance exercise, particularly in populations (i.e. athletes) that are resistant to highly invasive procedures (i.e. skeletal muscle biopsy).

2.7.2 Gene Expression in Skeletal Muscle

Skeletal muscle demonstrates a high level of plasticity, and distinctive differences in muscle phenotype are observed in response to chronic exposure to specific modalities of exercise. Acute bouts of exercise are known to produce transient changes in skeletal muscle gene expression which typically return to baseline levels within 24 hours after the cessation of exercise (Goldspink, 2003; Yang et al., 2005). However, chronic adaptations that occur at the level of the muscle are likely to arise due to the summation of many single (acute) bouts of exercise which lead to a cumulative, global alteration in gene and protein expression (Pilegaard et al., 2000; Stepto et al., 2009). Ultimately the balance between mRNA synthesis and degradation will dictate the balance between protein synthesis and degradation which will determine if phenotypic changes take place within skeletal muscle (Coffey et al., 2007a; Lai et al., 2010). Therefore, exercise can be viewed as an upstream modifiable factor that activates a downstream cascade of events leading to functional adaptation/s (refer to Figure 2-8). In terms of specific adaptations, strength training has been shown to have minimal effect on mitochondrial biogenesis or patterns of substrate metabolism (Stepto et al., 2009). However, this modality of exercise has been shown to up-regulate genes that promote protein synthesis, transcription, translation and hypertrophy, thus maintaining the phenotype for improved muscle force production (Coffey et al., 2007b; Deldicque et al., 2008; Dennis et al., 2008; Mahoney et al., 2008; Spiering et al., 2008; Stepto et al., 2009; Yang et al., 2005).

The majority of gene expression data relating to resistance exercise has been obtained via the percutaneous needle biopsy procedure (Bergström, 1975). A skeletal muscle biopsy can provide a great deal of localized histological, metabolic and molecular information relating to acute physiological responses and long-term adaptations to exercise. However, biopsy-induced muscle trauma can also induce significant changes in gene expression that can be mistaken for exercise-induced muscle damage (Nedergaard et al., 2007). Furthermore, the highly invasive nature of this research technique can be a major deterrent for high-performance athletes. Thus, the majority of physiological research pertaining to the molecular
responses to resistance exercise has been performed in untrained and/or recreationally individuals.

There is still much to be learnt about the time course of resistance exercise-induced gene expression. From the existing literature it is evident that acute bouts of resistance exercise

**Figure 2-8:** Proposed responses of a physiological system to an exercise stimulus. Manipulation of the modifiable factors will determine the downstream events which may lead to a functional adaption of the system. Adapted and modified from Spiering et al. (2008).
induce a rapid and persistent cellular stress response which is characterized by the upregulation of a number of inflammatory cytokines/chemokines (i.e. IL-6, IL-8, TNF-α, MCP-1), and heat shock proteins (i.e. HSP70, HSF1) (Deldicque et al., 2008; Louis et al., 2007a; Nieman et al., 2004; Paulsen et al., 2007). In addition, an increase in the mRNA expression of a number of metabolic (i.e. CD36, CPT1, HKII, PDK4), proteolytic (i.e. MuRF-1, MAFbx), myogenic (i.e. Myo-D, myogenin, Myf-5), and mechanical-related genes (i.e. CARP) generally peaks 3-8 h post-exercise (Bickel et al., 2005; Coffey et al., 2007a; Louis et al., 2007a; Mascher et al., 2008; Nedergaard et al., 2007; Yang et al., 2005; 2006). Thus, it is common practice for investigators to obtain tissue samples during a 3 h post-exercise window as a number of transcriptional and translational responses return to basal levels within 24 hrs of the exercise stimulus (Coffey et al., 2006a; Mahoney et al., 2008; Nedergaard et al., 2007; Pilegaard et al., 2000; Yang et al., 2005).

In response to acute bouts of damaging resistance exercise protocols (i.e. maximal eccentric contractions), a localized increase in inflammatory cytokines occurs within the injured muscle (Clarkson et al., 2002; Peake et al., 2005a). This pro-inflammatory response (refer to Section 2.8) is driven by skeletal muscle-derived fibroblasts, macrophages and myogenic precursor cells which may also be actively involved in the process of mononuclear cell activation and recruitment from the blood stream (Arnold et al., 2007; Chazaud et al., 2003; De Rossi et al., 2000; Ratajczak et al., 2003b; Tidball, 2005; Warren et al., 2004). The chemoattraction of circulating inflammatory cells to the site of tissue damage is a critical step in the subsequent repair and regeneration process of damaged skeletal muscle (Arnold et al., 2007).

However, repeated exposure to a similar exercise protocol results in an attenuated response with regard to muscle damage and protein degradation parameters; this phenomenon is termed the “repeated-bout-effect”, (Nedergaard et al., 2007; Stepto et al., 2009). Three major proteolytic systems exist within the human body: the lysosomal, the calcium-dependent, and the ubiquitin-proteasome system, with the latter two being responsible for the degradation of the intracellular and contractile proteins of skeletal muscle (Nedergaard et al., 2007). Atrogin-1 (also known as MAFbx), and MuRF-1 are key ubiquitin ligase proteins that are upregulated during skeletal muscle atrophy (Churchley et al., 2007). MuRF-1 has also been shown to be upregulated in response to an acute bout of resistance exercise in untrained adults (Mascher et al., 2008; Nedergaard et al., 2007; Yang et al., 2006). However, prior to a
second bout of resistance exercise in untrained adults, pre-exercise levels of MuRF-1 have been reported to be ~50% lower than that of the initial exercise bout (Nedergaard et al., 2007). In addition, the exercise-induced upregulation of MuRF-1 has been shown to be reduced by ~30%, when compared to the initial session, with only minor additional increases in enzymes regulating protein synthesis (Mascher et al., 2008). These results are supported by previous investigations demonstrating that prior training experience results in a lesser metabolic disturbance and an attenuated proteolytic signalling response resulting in the maintenance and/or enhancement of net protein balance (Coffey et al., 2006b; Nedergaard et al., 2007; Phillips et al., 1997; 1999).

Highly trained strength and power athletes require a far greater overload stimulus to induce significant increases in kinase activation and mRNA abundance when compared with nonhabitual resistance trainers (Coffey et al., 2006a; 2006b). Thus, it is common for these individuals to perform multiple training sessions within the same day (refer Section 2.3.3: Application and Variation of Training Load). However, there does appear to be a fine line between ‘undertraining’ and ‘overtraining’ both of which may lead to under-performance and/or maladaptation. For example, Coffey et al. (2007b) reported a marked suppression in IGF-1 transcription and Akt phosphorylation in conjunction with increased activity of protein-degradation pathways in response to a high-frequency resistance exercise protocol (i.e. four bouts of 3 x 10 repetitions of squat exercise at 75% of 1RM, separated by 3 hrs of recovery). Cumulatively, these findings identify an interesting adaptation paradigm for strength and power athletes. It is possible that chronically strength trained individuals experience a change in “genetic set point” (Stepto et al., 2009) which enables the transcriptional activity of myogenic genes to respond positively to frequent and intense resistance exercise. However, further research is required to elucidate such responses in well-trained individuals to determine the optimal frequency of high-intensity loading.

2.7.3 Influence of Feeding on Exercise-Induced Gene Expression

In addition to their function as a source of fuels and co-factors, micro and macronutrients also influence gene and protein expression (Müller et al., 2003; Pégorier et al., 2004). The molecular structure of a particular nutrient will determine the specific signalling pathway/s that will be activated and changes in nutrient structure or availability can have profound
effects on the transcriptional response to exercise (Bouwens et al., 2007; Müller et al., 2003). For example, van Erk et al. (2006) demonstrated that 317 and 919 genes in peripheral blood were differentially expressed following either a high-carbohydrate (46 g carbohydrate, 19.3 g protein, 14.4 g fat) or a high-protein breakfast (13.9 g carbohydrate, 57.2 g protein, 12.2 g fat), respectively, when compared with fasted, pre-breakfast levels.

Carbohydrate ingestion prior to and during intense aerobic exercise has been shown to result in a diminished pro- and anti-inflammatory cytokine response as determined by mRNA and plasma protein concentrations (Braun et al., 2004; Nieman et al., 1998; 2005a). Conversely, investigations into the influence of carbohydrate ingestion on the cytokine response to resistance exercise have produced equivocal results (Koch et al., 2001; Nieman et al., 2004). For example, Nieman et al. (2004) demonstrated that the ingestion of carbohydrate or a placebo during a 2 h intensive resistance exercise session did not influence the exercise-induced mRNA responses for IL-6, IL-10, IL-1ra in plasma or IL-1β, IL-6, IL-8 and TNF-α in muscle.

Growing evidence suggests that commencing aerobic exercise with low-muscle glycogen content enhances the transcription rate of a number of metabolic genes involved with aerobic training adaptation (Febbraio et al., 2002; Keller et al., 2001; Pilegaard et al., 2002). Major metabolic adaptations to aerobic exercise include a slower utilization of muscle glycogen and blood glucose and a greater reliance on fat oxidation during exercise of a given intensity (Hawley, 2002; Holloszy et al., 1984). Thus, during the repeated submaximal contractions that are performed during sustained aerobic exercise, mitochondria rich type I fibers have an enhanced ability to access alternative fuel sources to sustain exercise (Hawley, 2002). However, the same cannot be said in the context of resistance exercise which requires the recruitment of the larger, glycogen-dependent type II fibers during the intermittent, moderate- to high-intensity contractions (Tesch et al., 1984; 1986). Performing resistance exercise in a glycogen-depleted state has been shown to attenuate key cellular pathways (i.e. extracellular signal-regulated kinase and Akt intracellular signalling pathways) implicated in cellular growth and development (Churchley et al., 2007; Creer et al., 2005). These responses arise due to the fact that glycogen depletion results in a greater reliance on lipid and protein metabolism in an attempt to maintain cellular energy production (Pilegaard et al., 2000; Pilegaard et al., 2002). Such responses are clearly detrimental to the muscular adaptations (i.e. increase myofibrillar content) that are sought after by strength and power athletes.
Although post-exercise feeding is known to augment skeletal muscle glycogen and protein synthesis (Drummond et al., 2009a; Hawley et al., 2010; Phillips et al., 1997), the influence of post-exercise feeding on the cytokine response to high-power, HIRE in well-trained adults has not been investigated. Such research will enable a greater understanding of the modifiable factors that have the potential to influence various muscle repair and regeneration pathways in well-trained adults (refer to Section 2.8).

2.8 Chemokine Responses to Resistance Exercise

Chemokines, also known as chemotactic cytokines, are small-molecular-weight proteins (70-90 amino acids) that influence leukocyte migration in a concentration-dependent fashion (Baggiolini, 1998; Epstein et al., 1998; Moser et al., 2001; Rollins, 1997; Warren et al., 2004). Furthermore, chemokines play a pivotal role in host defense mechanisms and have the ability to influence angiogenesis, proliferation of hematopoietic precursors, and collagen production (Kunkel, 1999; Zlotnik et al., 2000). More than 50 chemokines and 18 chemokine receptors are currently included in the human chemokine system (Moser et al., 2001). Four chemokine subfamilies (C, CC, CXC, CX_{3}C) exist based upon the position of one or two cysteine residues near the NH_{2}-terminal end; the CC and CXC chemokines are the most abundant (Epstein et al., 1998; Warren et al., 2004). Each subfamily has a slightly different chemotactic function and further classification can distinguish between “inflammatory” and “homeostatic” chemokines (refer to Table 2-3). Inflammatory chemokines influence leukocyte trafficking during inflammation and tissue injury whereas homeostatic chemokines navigate leukocytes to and within secondary lymphoid organs and bone marrow (Deshmane et al., 2009).

Chemokines of the CC family are predominately involved in the migration and activation of lymphocytes, monocytes and macrophages (Warren et al., 2004). As such, a number of inflammatory diseases with mononuclear cell involvement (i.e. rheumatoid arthritis, asthma and atherosclerosis) have been associated with elevated CC chemokine expression (Aukrust et al., 2001; Kaur et al., 2006; Patel et al., 2001). Furthermore, an enhanced expression of CC chemokines is also evident within injured skeletal muscle (Hirata et al., 2003; Summan et al., 2003b). Members of the CXC family also attract mononuclear cell populations, along with polymorphonuclear neutrophils (Martin et al., 2003; Nagase et al., 2002; Warren et al., 2004),
and are involved in the processes of tissue inflammation and angiogenesis (Belperio et al., 2000; Romagnani et al., 2004).

**Table 2-3:** A functional classification of human chemokines and chemokine receptors involved with lymphocyte trafficking. Table adapted from Moser et al. (2001).

<table>
<thead>
<tr>
<th>Role</th>
<th>Chemokines</th>
<th>Receptor</th>
</tr>
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<tbody>
<tr>
<td>Homeostatic</td>
<td>CTACK, MEC</td>
<td>CCR10</td>
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<td></td>
<td>SDF-1</td>
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<td>BCA-1</td>
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<td>SLC, ELC</td>
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<td></td>
<td>TECK</td>
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<td></td>
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<td>Dual</td>
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<td>LARC</td>
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<td>Inflammatory</td>
<td>I-TAC, MIG, IP10</td>
<td>CXCR3</td>
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<td>CXCL16</td>
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<td></td>
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<td>CCR1</td>
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<td>MCP-1, MCP-2, MCP-3, MCP-4</td>
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<td>Eotaxin-1, Eotaxin-2, Eotaxin-3, RANTES, MCP-2, MCP-3, MCP-4, MEC</td>
<td>CCR3</td>
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<td>CCR8</td>
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<td>Fractalkine</td>
<td>CX3CR1</td>
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Specific seven-transmembrane spanning G-protein-coupled receptors mediate the biological effect of chemokines (refer to Table 2-3) (Horuk, 2001). In most instances, each chemokine receptor has a relative affinity for different chemokines (CXCR4 is an exception to this, refer to Section 2.8.1 and 2.8.2) and one ligand may also stimulate multiple receptors (Horuk, 2001; Kucia et al., 2004b). Chemokine receptor binding initiates a cascade of intracellular events that begins with the ligation of the receptor by its high affinity ligand (Epstein et al., 1998; Horuk, 2001). The resulting conformational change leads to a disassociation of heterotrimeric G proteins into α and βγ subunits which act as second messengers in receptor signalling (Epstein et al., 1998; Horuk, 2001). The ensuing signalling cascade includes the
activation of various effector enzymes which leads to increases in intracellular Ca\textsuperscript{2+} and activation of protein kinases (Horuk, 2001; Tilton et al., 2000). In the following sections, the chemokines that play pivotal roles in inflammatory and regenerative pathways within skeletal muscle will be discussed as they may also contribute to the early recovery processes following HIRE.

### 2.8.1 C-X-C Chemokine Receptor Type 4 (CXCR4)

CXCR4 is one of the most studied chemokine receptors due to its involvement in the regulation and migration of different types of stem/progenitor cells, organogenesis, hematopoiesis, immune response, HIV entry and metastasis of a variety of cancers (Busillo et al., 2007; Feng et al., 1996; Miller et al., 2008; Zlotnik, 2006). Furthermore, CXCR4 also has tissue specific roles (Miller et al., 2008) which include axon guidance cueing during the development of the nervous system (Chalasani et al., 2003). Due to its many and diverse roles, CXCR4 is functionally expressed on a number of cell types including hematopoietic progenitor cells, skeletal muscle satellite cells, most leukocyte subsets including T-lymphocytes, B cells and monocytes (but only weakly expressed on natural killer cells), endothelia and epithelia cells (Förster et al., 1998; Murdoch, 2000; Ratajczak et al., 2003b; Volin et al., 1998).

Nuclear Respiratory Factor-1 (NRF-1) has been identified as the major transcription factor that positively regulates the basal transcription of CXCR4 (Wegner et al., 1998). Furthermore, CXCR4 expression can increase as a result of intracellular second messengers such as calcium and cyclic-AMP, various interleukin cytokines and growth factors (e.g. vascular endothelial growth factor) (Busillo et al., 2007; Cristillo et al., 2002; Salcedo et al., 1999). Conversely, inflammatory cytokines such as tumor necrosis factor-\(\alpha\), interferon-\(\gamma\) and IL-1\(\beta\) attenuate CXCR4 expression (Busillo et al., 2007).

Intense aerobic exercise (e.g. 70-85% VO\textsubscript{2max}) and high-volume, large muscle mass, resistance exercise protocols initiate a biphasic response in leukocyte count (Pedersen et al., 1998). Immediately post-exercise, the total leukocyte count can increase 50-100% above baseline values and this is referred to as “acute leukocytosis” (Natale et al., 2003; Paulsen et al., 2005; Simonson et al., 2004a). However, within 30 min of recovery, leukocyte counts
may decline to 30-60% below baseline values (acute lymphopenia) (Risøy et al., 2003). The magnitude and duration of the leukocyte response to exercise is highly dependent upon the exercise mode, volume, intensity, duration and the rest period length (Mayhew et al., 2005; Nieman et al., 1992; Risøy et al., 2003).

Increased glucocorticoid activity has been associated with aerobic exercise-induced lymphopenia (Deuster et al., 1988; Okutsu et al., 2005; Shinkai et al., 1996). More specifically, aerobic exercise-induced increases in cortisol have been shown to up-regulate the expression of CXCR4 on T-lymphocytes in a dose-dependent manner which may modify lymphocyte trafficking (Nagatomi et al., 2002; Okutsu et al., 2002; 2005). Furthermore, administration of a glucocorticoid receptor antagonist (RU-486) attenuates CXCR4 expression which confirms the positive influence of the glucocorticoid receptor pathway on CXCR4 augmentation (Okutsu et al., 2005). However, following HIRE protocols that incorporate long-rest periods, no significant correlations have been reported between cortisol levels and post-exercise leukocyte counts (Kraemer et al., 1996; Mayhew et al., 2005; Nieman et al., 2004). Furthermore, it is evident that the transient immune changes to such resistance exercise protocols are modest compared to those measured after intense and sustained aerobic exercise and generally return to baseline levels within 2-3 h post-exercise (Mayhew et al., 2005; Natale et al., 2003; Nieman et al., 2004; Simonson et al., 2004b; Tvede et al., 1989). Since no association exists between cortisol secretion and alterations in leukocyte populations following HIRE protocols, other physiological events must be responsible for the transient changes in leukocyte trafficking.

2.8.2 Stromal-Cell Derived Factor-1 (SDF-1)

Unlike other chemokine receptors that possess the ability to bind multiple ligands, CXCR4 selectively binds to Stromal Cell-Derived Factor-1 (SDF-1) which is also known as CXCL12 (Busillo et al., 2007). SDF-1 is unique to other CXC chemokines as it resides on chromosome 10, whereas all other CXC chemokines reside on chromosome 4 (Shirozu et al., 1995). Unlike most other chemokines whose expression usually occurs in response to pro-inflammatory conditions, there is little evidence indicating that SDF-1 is responsive to inflammatory or immunological stimuli (Nagase et al., 2002). Although SDF-1 is a potent chemoattractant of lymphocytes and monocytes in vivo, it appears that its role is more directed towards the basal extravasation of these cell populations rather than in inflammatory
responses (Bleul et al., 1996; Gupta et al., 1999). Thus, SDF-1 is classified as “homeostatic” chemokine (Nagase et al., 2002) that is expressed constitutively in a number of tissues and cell lines including bone marrow fibroblasts, cardiac tissue, skeletal muscle, liver, neural tissue and the kidneys (Kucia et al., 2004).

However, following tissue injury (e.g. myocardial infarction, irradiation, hypoxia or toxic agent), SDF-1 expression/secretion is increased in the affected tissues thereby enhancing the responsiveness of circulating CXCR4⁺ cells (Kucia et al., 2004b; Ratajczak et al., 2006). As CXCR4 is a marker for hematopoietic, endothelial, neural, muscle and liver stem cells (Kucia et al., 2004; Miller et al., 2008), an SDF-1 induced increase in the chemoattraction of the relevant cell populations to the site of damage enhances the potential for repair and regeneration (Kucia et al., 2004b; Ratajczak et al., 2006). In addition, Griffin et al. (2010) recently demonstrated that the SDF-1/CXCR4 pairing is necessary for the proper fusion of muscle cells during myogenesis and also regulates the migration of proliferating and terminally differentiated skeletal muscle cells.

To date, the SDF-1/CXCR4 signalling axis has not been exclusively examined in response to resistance exercise or exercise-induced muscle damage models. Following an acute bout of supra-maximal anaerobic exercise (1,000 m of rowing ergometry), Morici et al. (2005) reported no change in the plasma concentrations of SDF-1. Although these limited findings are in accordance with the belief that SDF-1 is a “homeostatic” chemokine (Nagasawa et al., 1998; Nagase et al., 2002), they do not rule out the possible involvement of progenitor cell homing to skeletal muscle as tissue levels of SDF-1 were not measured by Morici et al. (2005). Furthermore, as exercise intensity increases, oxygen availability becomes compromised and low oxygen concentrations have been shown to increase the expression of CXCR4 in various cell types (Schioppa et al., 2003; Wahl et al., 2008). Thus, a hypoxia-induced increase of CXCR4 on stem cells may increase their chemotactic responsiveness to tissue-specific SDF-1 secretion. Such a response would enhance the potential for tissue repair and regeneration following exercise. Further investigation is required to ascertain whether or not the SDF-1/CXCR4 signalling axis plays a significant role during the acute response to exercise protocols that have the potential to elicit muscle damage.
Macrophage inflammatory protein-1β (MIP-1β) is also known as CC chemokine ligand 4 (CCL4) (Bacon et al., 2002). MIP-1β exclusively binds to the CCR5 receptor which is expressed in a number of cells including macrophages, monocytes, T-lymphocytes, natural killer cells, hematopoietic progenitors and dendritic cells (Menten et al., 2002; Rollins, 1997). Thus, MIP-1β exerts a potent chemotactic influence over these cell populations during various physiological settings. In addition to playing a critical role in leukocyte trafficking, MIP-1β also induces the synthesis of other pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α in fibroblasts and macrophages (Connolly et al., 2004b).

Lymphocyte migration from blood into tissue is dependent upon the integrin-mediated adhesion of lymphocytes to the endothelium (Tanaka et al., 1993). Chemokines activate the surface bound integrins on leukocytes and MIP-1β augments T lymphocyte adhesion to vascular cell adhesion molecule 1 (VCAM-1) (Tanaka et al., 1993). However, in some disorders, the abnormal accumulation of leukocytes can contribute to the pathogenesis of the disease and the presence of MIP-1β has been associated/observed in a number of disorders (Menten et al., 2002) including rheumatoid arthritis (Patel et al., 2001), Alzheimer’s disease (Xia et al., 1999), bacterial meningitis (Lahrtz et al., 1998) and atherosclerosis (Aukrust et al., 2001). In addition, gene expression profiling has demonstrated that enhanced expression of CC chemokines, including MIP-1β, is a characteristic feature of injured skeletal muscle due to their roles in attracting inflammatory cells types (i.e. macrophages) to the site of tissue damage (Hirata et al., 2003; Summan et al., 2003b; Warren et al., 2004). For example, Hirata et al. (2003) reported a 46.6-fold increase in MIP-1β mRNA levels within mouse skeletal muscle following cardiotoxin injury.

It is now believed that blood-borne macrophages play a greater role in muscle regeneration that goes beyond phagocytosis as macrophages have been shown to regulate the proliferation and differentiation of skeletal muscle satellite cells (Cantini et al., 1994; 1995; Lescaudron et al., 1999; Robertson et al., 1993). Therefore, therapeutic interventions that target the recruitment of macrophages to sites of injury are becoming increasingly popular. For example, muscle transplants that were conditioned with MIP-1β and VEGF demonstrated accelerated and augmented monocyte and macrophage infiltration and satellite cell differentiation and/or proliferation when compared to controls (Lescaudron et al., 1999). Both
MIP-1β and VEGF enhanced muscle regeneration within transplanted tissues and importantly, these regenerative processes never preceded monocyte-macrophage infiltration (Lescaudron et al., 1999). However, as cultured myoblasts exposed to MIP-1β have demonstrated an increased proliferative response, it is evident that CC chemokines that are expressed within injured tissue are also capable of acting directly on the muscle cells (Yahiaoui et al., 2008). These results indicate that MIP-1β has a novel role in muscle repair and regeneration beyond leukocyte trafficking.

Exercise-induced muscle damage produces an inflammatory response that is similar to that seen in muscle trauma patients (Ostrowski et al., 1998), whereby neutrophils and macrophages invade muscle tissue in large numbers (Nieman et al., 2005b; Tidball, 2005). However, despite the similarities in the inflammatory processes, the response of plasma MIP-1β to exercise has produced some equivocal results. Plasma MIP-1β concentrations have been reported to increase by 4.1-fold (Ostrowski et al., 2001) and 1.2-fold (Nieman et al., 2005b) following a marathon (42.2km) and ultra-marathon (160km) events, respectively. However following exhaustive, prolonged treadmill running (2.5 h at 75% \( \text{VO}_2\max \)), no significant changes in plasma MIP-1β were reported in endurance-trained men (Ostrowski et al., 1998). In support of this lack of MIP-1β response to exercise, Risøy et al. (2003) also reported no change in plasma MIP-1β following a HIRE protocol involving back squats, front squats and knee extensions performed at 100% of 6RM. Furthermore, 300 unilateral, maximal isokinetic eccentric contractions (30°/sec) of the quadriceps were also shown to produce no significant change in plasma MIP-1β immediately, 30 min, 1 h, 6 h, and 23 h post-exercise (Paulsen et al., 2005). It is possible that the duration of resistance exercise in the aforementioned studies (35-60 min in total) failed to meet the “volume/duration threshold” required to elicit a noticeable change in circulating plasma MIP-1β (determined via routine ELISA methods). However, more recently, Mathers et al. (2012) reported a significant 2 h post-exercise increase in skeletal muscle MIP-1β mRNA expression levels (determined via qPCR) following 3 sets of 12 repetitions of maximal unilateral knee extensions in elderly (67.2 ± 1.5 years) participants. As chemokine gene expression is greatest during the early recovery from exercise (Mathers et al., 2012), further investigation is required into the transcriptional response of MIP-1β following intense resistance exercise protocols that are common to high performance strength and power athletes. Such research would shed more light on the potential role of acute inflammatory processes in long-term adaptation to resistance exercise.
2.8.4 Monocyte Chemotactic Protein-1 (MCP-1)

Monocyte chemotactic protein-1 (MCP-1), also known as CCL2, was the first CC chemokine identified (Deshmane et al., 2009). MCP-1 is produced by a number of cell types including monocytes, macrophages, skeletal muscle satellite cells, endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytes and microglial cells either constitutively or following induction by oxidative stress, tissue injury, cytokines or growth factors (Chazaud et al., 2003; De Rossi et al., 2000; Deshmane et al., 2009; Harmon et al., 2010). MCP-1 exerts its effects through the CCR2 receptor which has two alternatively spliced forms; CCR2A and CCR2B (Deshmane et al., 2009). CCR2A is the major isoform expressed by mononuclear cells and vascular smooth muscle cells (Bartoli et al., 2001), whereas CCR2B is predominately expressed by monocytes and activated NK cells (Deshmane et al., 2009). Due to the diverse expression of the CCR2 receptor, MCP-1 plays a role in the induction and migration of various immune cells during routine immunological surveillance of tissues, as well as in response to inflammation (Henningsen et al., 2011; Uguccioni et al., 1995). In addition, MCP-1 levels are also increased in a localized fashion and within the circulation following traumatic injury or exercise-induced muscle damage (Chazaud et al., 2003; Paulsen et al., 2005; Peake et al., 2005b; Summan et al., 2003a, b; Yahiaoui et al., 2008). As a result, circulating lymphocytes and monocytes that are expressing the associated CCR2 receptor are attracted to areas of tissue damage (Fantuzzi et al., 1999; Loetscher et al., 1994; Uguccioni et al., 1995). Monocytes exhibiting inflammatory profiles infiltrate the damaged tissue to aid in phagocytosis (Arnold et al., 2007). Following the removal of cellular debris, the inflammatory monocytes are then converted to anti-inflammatory macrophages that stimulate myogenesis and muscle fiber growth (Arnold et al., 2007). During injury, MCP-1 has also been shown to be colocalized with resident macrophages and satellite cell populations (Hubal et al., 2008). These finding suggest that satellite cells are either “primed” to produce more MCP-1 or more MCP-1 protein is chemotactically attracted to satellite cells during tissue injury (Chazaud et al., 2003; De Rossi et al., 2000; Hubal et al., 2008; Warren et al., 2005).

Previous evidence has demonstrated that MCP-1 is highly responsive to damaging exercise protocols. For example, MCP-1 levels are greater following eccentric-only muscle contractions, when compared to concentric-only contractions, and circulating levels have been shown to peak at ~6 h post-exercise (Hubal et al., 2008; Paulsen et al., 2005). Furthermore, following intense aerobic exercise, plasma MCP-1 levels have been correlated
with indices of muscle damage; creatine phosphokinase levels ($r = 0.49$) and post-exercise delayed onset of muscle soreness scores across 7 days ($r = 0.28 - 0.77$) (Nieman et al., 2005b). However, other factors such as exercise intensity and circulating cortisol have also been shown to positively influence the plasma concentrations of MCP-1 and the expression of the associated CCR2 receptor on monocytes, respectively (Okutsu et al., 2008; Peake et al., 2005b). Thus, it appears that the recovery from intense exercise is associated with an upregulation of MCP-1/CCR2 expression to aid with subsequent tissue repair and regeneration processes.

As mentioned in Section 2.7.2, repeated exposure to a similar exercise protocol results in a “repeated-bout-effect” whereby muscle damage and protein degradation parameters are attenuated (Nedergaard et al., 2007; Stepto et al., 2009). However, in response to a second bout of 300 maximal eccentric contractions, Hubal et al. (2008) reported a greater post-exercise (6 h) MCP-1 transcriptional response (12.2-fold increase) when compared to the first bout of the same exercise (9.6-fold increase) performed 4 weeks earlier. In addition, a greater percentage of satellite cells were colocalized with the MCP-1 protein after the second bout of exercise (54%), when compared with the first (29%), indicating an enhanced interaction with myogenic pathways post-exercise (Hubal et al., 2008). The increased transcriptional and translational responses of MCP-1 coincided with a faster recovery of strength measures following the second bout of eccentric exercise which supported the repeated-bout effect. As satellite cells communicate with other cell populations via chemokine secretion, it appears that altered communication between cell populations via an upregulation of MCP-1 mRNA, in conjunction with an increased expression of MCP-1 protein by satellite cells, contributes to a faster recovery of strength following damaging exercise.

There is growing evidence that MCP-1 plays a pivotal role in determining muscle phenotypes (Harmon et al., 2010). For example, Warren et al. (2005) demonstrated that CCR2 knockout mice exhibit impaired muscle recovery following injury, increased fat infiltration and an accumulation of fibrotic tissue within skeletal muscle. Furthermore, eight genetic variants in the MCP-1 and CCR2 genes have been associated with initial measures of skeletal muscle strength and the strength response to resistance exercise in men and women (Harmon et al., 2010). As MCP-1 is an important signalling component in the communication between satellite cells and surrounding cell populations, further insight into the MCP-1 response to resistance exercise may contribute to the development of novel post-exercise recovery
strategies. Such research will have practical implications to strength and power athletes who regularly perform multiple HIRE sessions, involving the same major muscle groups, within the same day.
3.0 RESEARCH AIMS AND EXPERIMENTAL APPROACH
3.1 Specific Aims

The major goal of this thesis was to determine and examine, at multiple levels, the physiological variables that contribute to the early recovery and adaptive processes following high-power, HIRE in competitive weightlifters and resistance-trained adults.

To achieve this, three separate research projects were done, each of which contained a systematically controlled and monitored HIRE intervention in healthy, competitive weightlifters and/or resistance-trained adults. Of specific interest were the influence of variations in training load (Study 1) and the effects of post-exercise feeding (Study 3) on the exercise-induced transcriptional responses in competitive weightlifters and resistance-trained adults, respectively. Furthermore, as competitive weightlifters routinely perform multiple HIRE sessions within the same day, I sought to examine and compare the skeletal muscle structural and functional responses to “double-day training” between competitive weightlifters and resistance-trained adults (Study 2). Thus, a multi-disciplinary approach was taken to examine the acute exercise-induced responses to high-power, HIRE from a variety of scientific perspectives. These perspectives included alterations in performance, systemic physiology, psychology and skeletal muscle structure and function.

3.2 Experimental Approach

STUDY 1: TRANSCRIPTIONAL, OXIDATIVE STRESS AND PERFORMANCE RESPONSES TO VARIATIONS IN TRAINING LOAD IN COMPETITIVE WEIGHTLIFTERS

The purpose of this investigation was to identify and examine systemic responses that are associated with the recovery from and adaptation to prescribed, short-term variations in training load in competitive weightlifters. To achieve this, the gene expression, protein oxidation, performance and psychological responses to 2 wk of overload (lifting 36,508 ± 11,299 kg/wk) and 1 wk of recovery training (lifting 27,056 ± 8,442 kg/wk) were documented in seven International-level competitive weightlifters. Venous blood samples were obtained on seven occasions throughout the study period for the purpose of genomic and protein oxidative analyses. Performance testing was conducted on a weekly basis to assess changes in the physical response to the prescribed variations in training load. In
addition, psychological questionnaires were completed on a daily basis to assess changes in the affective response to the training program. By systematically controlling and monitoring the increases and decreases in training stress, I sought to identify factors that are associated with the anticipated restoration and/or improvement in performance following a short-term recovery period in competitive weightlifters.

**STUDY 2: DIVERGENT MUSCLE FUNCTIONAL AND ARCHITECTURAL RESPONSES TO TWO SUCCESSIVE HIGH INTENSITY RESISTANCE EXERCISE SESSIONS IN COMPETITIVE WEIGHTLIFTERS AND RESISTANCE TRAINED ADULTS**

To test the hypothesis that competitive weightlifters posses an enhanced ability to rapidly recover neuromuscular function between successive HIRE bouts that are performed on the same day, sixteen competitive weightlifters and sixteen resistance-trained adults performed two equivalent HIRE sessions separated by 4–6 h rest. Isometric front squat peak force, contractile rate of force development and contractile impulse were determined before and after each training session. Furthermore, to determine if acute changes in muscle function were associated with acute changes in muscle architecture, the pennation angle, anatomical and physiological thickness of the vastus lateralis were also determined pre- and post-exercise.

**STUDY 3: PERIPHERAL BLOOD MONONUCLEAR CELL AND SKELETAL MUSCLE GENE EXPRESSION RESPONSES TO HIGH POWER, HIGH INTENSITY RESISTANCE EXERCISE AND FEEDING DURING RECOVERY**

The results of Study 1 indicated that the HIRE performed by competitive weightlifters induced a robust increase in CXCR4 mRNA expression in circulating PBMCs. Furthermore, it was evident that this form of training elicited a degree of exercise-induced muscle damage resulting in a significant immunological and inflammatory stress response. The purpose of this investigation was to examine the influence of high-power, high-intensity resistance exercise (HIRE), similar to that performed by competitive weightlifters, on the expression of the inflammatory chemokine/receptor pairings SDF-1/CXCR4 and MCP-1/CCR2. Furthermore, the effects of a post-exercise meal on the expression of the identified
chemokine/receptor pairings were also investigated. Seven resistance-trained adults took part in three experimental conditions; Trial A – Exercise and Meal, Trial B – Exercise and No Meal and, Trial C – No Exercise and No Meal (rest only control condition). Venous blood samples were obtained on four occasions (Baseline, Pre-Exercise, Post-Exercise (0 min and 3 h)) during each experimental condition for the purpose of genomic and serum analyses. In addition, a 3 h post-exercise skeletal muscle biopsy was obtained on all occasions for the purposes of genomic and protein analyses.
4.0 TRANSCRIPTIONAL, OXIDATIVE STRESS AND PERFORMANCE RESPONSES TO VARIATIONS IN TRAINING LOAD IN COMPETITIVE WEIGHTLIFTERS
4.1 Abstract

The purpose of this investigation was to identify and examine systemic responses associated with the recovery from and adaptation to prescribed, short-term variations in training load in competitive weightlifters. To achieve this, gene expression, protein oxidative, performance and psychological responses to 2 wk of overload (lifting 36,508 ± 11,299 kg/wk) and 1 wk of recovery (lifting 27,056 ± 8,442 kg/wk) training were investigated. Preliminary cDNA microarray analyses were performed on pre-exercise and 3 h post-exercise peripheral blood mononuclear cell (PBMC) samples obtained at the end of the 2 wk overload. In total, 202 genes were identified as being differentially expressed (≥1.4-fold change) and collectively presented a strong genetic signature of immunological and inflammatory stress. Follow up qPCR analyses on selected genes demonstrated that 2 wk of overload resulted in a greater upregulation (3 h post-exercise) of Chemokine (C-X-C motif) Receptor 4 (CXCR4), DNA-Damage-Inducible Transcript 4 (DDIT4) and Chemokine (C-C motif) Ligand 4 (CCL4) when compared to the recovery week. However, subsequent enzyme-linked immunosorbent assays showed decreases in CCL4 protein (MIP-1β) in plasma from pre- to 3 h post-exercise after overload (-33.2 ± 22.7%) and recovery (-55.9 ± 5.2%). Furthermore, the 2 wk of overload resulted in greater pre- (35.2 ± 23.2%) and immediate post-exercise (35.3 ± 10.8%) plasma protein carbonyl (PC) levels, indicative of enhanced tissue damage, compared to the 1 wk of recovery. Maximal snatch and vertical jump performance were impaired after the overload and returned to previous best competitive performance levels during the recovery week. Finally, the participants demonstrated a negative affective response to the 2 wk overload, as determined by increased signs and symptoms of stress and Total Mood Disturbance Scores. Collectively, this study has demonstrated that an exercise-responsive expression of CXCR4, CCL4 and DDIT4 occurs following 2 wk of overload, concomitant with increased tissue damage and decreased muscular performance in competitive weightlifters. Conversely, a 1 wk period of recovery training was sufficient for the resolution of tissue damage, inflammatory gene expression and the restoration of performance. These results implicate chemokine signalling in the early recovery response following high-power, HIRE overload training in competitive weightlifters.
4.2 Introduction

Competitive weightlifters epitomize strength and power athletes as they have demonstrated some of the highest absolute and relative peak power outputs reported in the literature (Garhammer, 1980, 1982, 1991, 1993). Such displays of human performance have resulted from intensive training programs that exceed evidence-based recommendations for improving muscular strength and power (Ratamess et al., 2009). The training structure of these athletes will generally follow a repeated pattern of 2-3 wk of intensified loading (‘overload’) followed by 1 wk of reduced loading (‘recovery’). During overload periods, the total weekly training volume (repetitions × sets × load) may be increased by as much as 100% above “normal” training levels (Fry et al., 1993; Pistilli et al., 2008; Warren et al., 1992). Conversely, during recovery periods, the total weekly training volume may be decreased by ~50 - 75%, relative to the preceding weeks of overload (Drechsler, 1998; Pistilli et al., 2008; Wilson et al., 2008). The primary ways in which variations in total weekly training volume will arise are through increases or decreases in the prescribed number of training sessions per week and the number of sets and/or repetitions performed within each session. Variations in the training intensity may also be applied but the percentage changes between overload and recovery weeks are relatively minor in comparison to the other manipulated program variables (i.e. training session frequency, number of sets and repetitions).

Following short-term periods of recovery (i.e. 1 wk), competitive weightlifters exhibit a restoration and/or improvement in performance (Crewther et al., 2010; Fry et al., 1993). Conversely, it may take between 2-5 weeks for the restoration and/or an increase in performance to occur in other athletes (e.g. swimmers, cyclists, American football players) following the cessation of overload periods ranging from 1-3 wk in duration (Fry et al., 1997; Halson et al., 2004; Moore et al., 2007; Stone et al., 1998). Thus, it is appears that weightlifters possess an enhanced ability to rapidly recover neuromuscular function and competitive performance following short-term periods (i.e. 2-3 wk) of overload. Previous investigations into the influence of variations in training volume in competitive weightlifters have quantified changes in circulating hormones and performance indices (Fry et al., 1993; 2000; Warren et al., 1992). Although such research provides valuable information regarding the neuroendocrine responses of these athletes, further investigation into other systemic and molecular responses may help to explain how competitive weightlifters adapt to their uniquely intensive training cycles.
Acute bouts of intense muscular exercise result in a rapid cytokine-mediated pro-inflammatory response within skeletal muscle (Peake et al., 2005a; 2005b; Pedersen et al., 2001). However, it is now known that relatively brief exercise also leads to substantial cellular gene expression changes in peripheral blood mononuclear cells (PBMC) (Carlson et al., 2011; Connolly et al., 2004b; Radom-Aizik et al., 2008; 2009b). PBMCs are essential mediators of cellular stress and inflammatory responses as they produce various cytokines, chemokines and growth factors (Radom-Aizik et al., 2007). Mounting evidence is demonstrating that PBMC-derived cytokines and chemokines play a critical role in the signalling cascades that regulate muscle repair, regeneration and subsequent growth following injury (Hirata et al., 2003; Kucia et al., 2004; Warren et al., 2004). In addition, it has been proposed that the expression of inflammatory cytokines plays a pivotal role with initial measures of muscular strength and the strength response to resistance exercise in men and women (Harmon et al., 2010). Currently, only one publication has examined the PBMC transcriptional response to resistance exercise in moderately-trained adults (Carlson et al., 2011). As changes in PBMC gene expression are informative of changes in systemic physiology, the main objective of this investigation was to identify and examine PBMC transcriptional responses in highly-trained competitive weightlifters following 2 wk of overload and 1 wk of recovery.

Recent advances in high-throughput technologies, such as cDNA microarrays, have enabled researchers to screen for novel exercise-responsive genes from within the whole genome. In general, such studies have characterized transcriptional responses in PBMCs representing key events in the recovery from, and adaptation to, various aerobic exercise protocols in untrained and/or recreationally trained individuals (Büttner et al., 2007; Connolly et al., 2004b). To date, only one PBMC microarray investigation has been performed following a 30 min, moderate-intensity, moderate-volume resistance exercise protocol in moderately trained adults (Carlson et al., 2011). Carlson et al. (2011) demonstrated that the greatest gene response occurred 2 h post-exercise and that the majority of the 259 differentially expressed genes were involved in pathways related to immune responses, inflammation and cellular communication. Although the above-cited studies succeed in furthering our understanding of the acute responses to exercise through the discovery of novel exercise-responsive genes, they each have limitations. Firstly, as an individual’s training status has been shown to influence the gene expression response to exercise (Coffey et al., 2006a; 2006b; Nedergaard
et al., 2007; Stepto et al., 2009), the results obtained do not necessarily reflect the transcriptional responses in highly-trained athletes. Secondly, the exercise protocols used in these investigations are not ecologically valid in the context of competitive athletes due to the comparatively low exercise volumes and intensities that are prescribed. However, it is evident that the use of microarray technology during real-life training periods in highly-trained athletes would be a meaningful and worthwhile research approach to help inform subsequent analyses on smaller clusters of genes that act on relevant signalling pathways.

The first objective of this investigation was to identify exercise-responsive “candidate genes”, via cDNA microarray analyses, following 2 wk of overload in competitive weightlifters. The second objective was to extend the analysis of selected candidate genes by quantifying their basal and exercise-induced transcriptional responses, via real-time quantitative PCR (qPCR), following short-term variations in training load (i.e. 2 wk of overload followed by 1 wk of recovery). In addition, I sought to determine if plasma chemokine (CCL4/MIP-1β), protein oxidative (protein carbonyl) and psychological responses were associated with the variations in training load and weightlifting performance.

It was hypothesized that: (1) the cDNA microarray analyses would provide a profile of genes that respond to high-power, high-intensity weightlifting training; (2) following the 2 wk overload, basal and immediate post-exercise CCL4/MIP-1β and protein carbonyl levels would be significantly higher when compared to the 1 wk recovery period; (3) performance decrements would occur in response to the 2 wk overload, whereas a restoration of performance would occur with the subsequent 1 wk of recovery; and (4) during the 2 wk of overload, decrements in performance would be associated with an increase in Total Mood Disturbance Scores (determined via the Profile of Mood States questionnaire) and an increase in the signs and symptoms of daily stress (determined via the Daily Analysis of Life Demands of Athletes questionnaire).
4.3 Methods

4.3.1 Participants

Seven International-level competitive weightlifters volunteered to participate in this repeated-measures within-subject design study. Participants were recruited via Participant Recruitment Flyers which were posted at local weightlifting clubs (refer to Appendix B). As a requirement of the study, all participants: 1) were free from acute or chronic injury at the time of data collection; 2) were not using any performance enhancing supplements (e.g. creatine) or banned substances (WADA, 2010); and 3) abstained from using anti-inflammatory medications (e.g. Voltaren). The International competitive experience of the participants ranged in quality from Oceania Championship to Olympic Games (2008 Beijing Games) level. Each participant provided written informed consent prior to commencing the investigation. All experimental procedures were performed in accordance with the University of Auckland Human Participant Ethics Committee (refer to Appendix B).

4.3.2 Experimental Design

Data was obtained from each participant during the first four weeks of a seven week training build up for a regional competition: Week 1 represented a baseline period (lifting 18,544 ± 5,995 kg/wk); Week 2 and 3 represented a two week ‘overload’ period in which the training load was systematically increased for each participant relative to Week 1 (Week 2 - lifting 35,937 ± 11,536 kg/wk; Week 3 - lifting 37,078 ± 11,949 kg/wk); Week 4 represented a ‘recovery’ period in which the prescribed training load for each participant was intentionally reduced relative to the overload period (lifting 27,056 ± 8,442 kg/wk) (Figure 4-1). At the end of the baseline training period, peripheral blood samples were obtained from the participants, all of whom were in a rested and fasted state. In addition, at the end of the overload and recovery training periods, peripheral blood samples were obtained before (in a rested and fasted state), directly after (0 min) and 3 h after an identical weightlifting session and a standardized meal (48 kJ/kg body mass per athlete: 59% carbohydrate, 36% protein, 5% fat). At the end of each training week, maximal vertical jump testing was conducted prior to the penultimate morning training session. In addition, a simulated competition approach
was taken to assess changes in weightlifting performance (snatch and clean and jerk) across the 2 wk of overload and 1 wk of recovery training.

At the end of the 2 wk overload period, cDNA microarrays were used to identify differentially expressed genes of interest in peripheral blood mononuclear cell (PBMC) samples. Follow up real-time quantitative polymerase chain reaction (qPCR) analyses were performed across multiple time points within the 4 wk experimental period to extend the analysis of the selected candidate genes. In addition, selected plasma assays were used to measure protein oxidative and inflammatory protein responses following the overload and recovery periods. Finally, all participants were required to complete two psychological questionnaires each day. Changes in perceived mood, motivation, mental and physical well-being in response to the training program were calculated from the completed questionnaires.

**Figure 4-1: Overview of the 4 week study design.**
4.3.3 Training Program

The 4 wk study period coincided with the start of a 7 wk training build up for a regional competition. The training program was designed and administered by the lead investigator (Adam Storey) who was also a National Coach and the Head Coach to the participants. Structured preparatory training was performed leading up to the study period and a preceding recovery training week served as a baseline from which the training variables were systematically increased and decreased accordingly as shown in Figure 4-2.

Figure 4-2: Percentage change in the weekly total load (kg) (■), total repetitions (♦) and mean training load (kg) (Δ), relative to the baseline week, during the two week overload (grey) and one week recovery training period.

During the baseline, overload and recovery periods, the training program consisted of core weightlifting movements (i.e. snatch, clean and jerk, power snatch and power clean) and other supplementary exercises (i.e. front squats, back squats, clean pulls, snatch pulls) (refer to Appendix D). To determine whether any physiological and/or performance changes were a result of the cumulative training stress (i.e. overload versus recovery), the training sessions were replicated on the performance testing (Fridays) and subsequent biological sample
collection days (Saturdays). In addition, as acute periods of calorie restriction incite distinctive genomic signatures (Bouwens et al., 2007; Crujeiras et al., 2008), a standardized mixed macronutrient pre- and post-exercise meal was provided to the athletes to ensure ecological validity within the current investigation.

For each participant, the total load (kg) per exercise within each training session was calculated using Equation 1 and the resultant loads were summated to give a total weekly load (kg). The mean weekly load (kg) for each participant was calculated using Equation 2.

**Equation 1:**

\[
\text{Total Load per Exercise (kg)} = \text{Repetitions} \times \text{Sets} \times \text{Load (kg)}
\]

**Equation 2:**

\[
\text{Mean Training Load (kg)} = \frac{\text{Total Load Week}^{-1}}{\text{Total Repetitions Week}^{-1}}
\]

### 4.3.4 Performance Testing

Performance testing consisted of; 1) maximal vertical jump prior to the start of the Friday morning training sessions (Week 1-4) and, 2) maximal snatch and clean and jerk in a simulated competition format during the Friday afternoon training sessions (Week 2-4) (refer to Figure 4-1).

**Vertical Jump Performance**

The vertical jump was chosen as a representative measure of muscular power due to its kinematic similarities to weightlifting movements (Canavan et al., 1996; Garhammer et al., 1992). In addition, strong correlations have been demonstrated between vertical jump performance and weightlifting ability (Carlock et al., 2004; Fry et al., 2006a).

Counter-movement vertical jump performance was measured using a Vertec® Vertical-Jump Tester (Sports Imports, Columbus, OH). All participants were familiar with using the device (Figure 4-3). Following a standardized warm up consisting of light jogging and calisthenics, the standing reach (cm) of each participant was measured by moving the highest possible
Vertec® vane from a standing position. Participants began the counter-movement jump trials in a standing position, rapidly descended to a required parallel thigh position and immediately performed an “explosive” upward movement with the intention of touching the highest possible Vertec® vane (Figure 4-3). Participants performed three maximal counter-movement vertical jump trials with 1 min rest between attempts. The participant’s vertical jump score (cm) was expressed as the difference between maximal jump reach and standing reach. The best score of the three attempts was recorded as their maximum for the given session. All participants remained blind to their vertical jump scores throughout the duration of the 4 wk study. Vertical jump peak power output was calculated using Equation 3 (Harman et al., 1991).

![Figure 4-3: Vertec vertical jump tester. The vertical jump score (cm) is expressed as the difference between maximal jump reach (1) and standing reach (2).](image)

**Equation 3:**

Peak Power Output (W) = (61.9 × jump height (cm)) + (36.0 × body mass (kg)) + 1822
Snatch and Clean and Jerk Performance

Due to the highly taxing nature of near maximal to maximal weightlifting movements, snatch and clean and jerk (C&J) testing was conducted only during the overload and recovery training period (refer to Figure 4-1). Therefore, each participant’s current personal best lifts performed in an official competition served as their “baseline” values from which comparisons were made. All personal best lifts were obtained within 2 months of the start of the 4 wk study. During the maximal testing, the snatch preceded the C&J as done in competition (refer to Section 2.2.1 and 2.2.2 for a thorough description of the snatch and C&J). The simulated competition approach to testing weightlifting performance has previously been validated in the literature (Crewther et al., 2010; McGuigan et al., 2004; Passelergue et al., 1995). Each participant performed a standardized general warm up consisting of light jogging, calisthenics and stretching. A standardized snatch specific warm up followed. Participants were required to reach a target weight of 90% of their current 1 repetition maximum (1RM), rounded to the nearest official weight increment, within 8 sets. Double repetitions were performed for the first 4 sets of the warm up and single repetitions were performed for the remaining 4 sets with a 2 min rest between sets (Table 4-1). Following the warm up, participants performed three near-maximal to maximal attempt snatches separated by a 2 min rest as done in competition. The prescribed warm up protocol, including the first attempt, was repeated on all testing occasions. Successful execution of each attempted lift was verified in accordance with the 2009 – 2012 International Weightlifting Federation Technical and Competition Rules (refer to Appendix F) by the lead investigator, a certified National-level weightlifting referee. Following a successful attempt, the weight of the barbell was incrementally increased by 2-5kg in order to attempt and/or achieve a maximal lift within the three attempts. However, in the event of an unsuccessful lift, the weight remained the same for the subsequent attempt. Following the completion of the snatch testing, participants rested for 10 min as done in competition. A standardized C&J specific warm up followed in the same format as that of the snatch and each participant performed 3 near-maximal to maximal-attempt C&Js. The assessment of the competitive lifts by trained weightlifters is highly reliable with coefficients of variations of 2.3 - 2.7% (McGuigan et al., 2004).
Table 4-1: An example snatch and clean and jerk specific warm up protocol for the simulated competition.

<table>
<thead>
<tr>
<th>Current 1RM Snatch:</th>
<th>144 kg</th>
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</thead>
<tbody>
<tr>
<td>90% of Current 1RM Snatch:</td>
<td>130 kg</td>
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<table>
<thead>
<tr>
<th>Warm Up</th>
<th>Sets × Repetitions</th>
<th>Load (kg)</th>
<th>% of 1RM</th>
</tr>
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<tr>
<td>1</td>
<td>1 x 2</td>
<td>60</td>
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<td>1 x 1</td>
<td>130</td>
<td>90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Simulated Competition</th>
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<th>% of 1RM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attempt 1</td>
<td>136</td>
<td>94</td>
</tr>
<tr>
<td>Attempt 2</td>
<td>141</td>
<td>98</td>
</tr>
<tr>
<td>Attempt 3</td>
<td>145</td>
<td>101</td>
</tr>
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</table>

**10 minute Rest Interval**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>90% of Current Personal Best Clean and Jerk:</td>
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</tr>
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<table>
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<th>Warm Up</th>
<th>Sets × Repetitions</th>
<th>Load (kg)</th>
<th>% of 1RM</th>
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<tbody>
<tr>
<td>Attempt 1</td>
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<td>94</td>
</tr>
<tr>
<td>Attempt 2</td>
<td>185</td>
<td>98</td>
</tr>
<tr>
<td>Attempt 3</td>
<td>189</td>
<td>101</td>
</tr>
</tbody>
</table>
4.3.5 Blood Sampling and PBMC Isolation

Baseline and pre-exercise blood samples were obtained from an antecubital vein of each participant (in a rested and fasted state), by standard venepuncture technique, and collected into two 8 mL BD Vacutainer™ CPT™ Sodium Citrate Tubes (Becton Dickinson, Heidelberg, Germany). Upon acquisition, the samples were mixed by gently inverting the tubes 5 times. Post-exercise blood samples were obtained in the same manner on two occasions; immediately post-exercise (0 min) and 3 h post-exercise and standardized feeding.

The purpose of the CPT™ tubes is for the collection of whole blood and the separation of mononuclear cells (i.e. lymphocytes and monocytes). Each tube contains a cell separation medium comprised of a polyester gel and a density gradient liquid. At each time point, the blood samples were processed within 15 min according to the manufacturer’s recommendations and the blood processing methods described by Affymetrix (Affymetrix, 2003). Immediately prior to centrifugation, the blood samples were remixed by inverting the tubes 8–10 times. Centrifugation was performed at 1,636 g for 25 min in a swinging bucket centrifuge at room temperature (18°C) with brake. The upper layer of plasma was carefully aspirated, aliquoted and frozen at -80°C until further analyses. The remaining cell layer was transferred to a sterile 15 mL conical centrifuge tube using a siliconized glass Pasteur pipette. Phosphate buffered solution (PBS) (0.1 M, pH 7.4) was then added to bring the volume to 15 mL and the cells and PBS were mixed by gently inverting the tube 5 times. Centrifugation was performed at 328 g for 15 min (with brake). The resulting supernatant was discarded and cell pellets were resuspended in 10 mL of PBS (0.1 M, pH 7.4). A final centrifugation step was performed at 328 g for 10 min (with brake). The resulting supernatant was carefully aspirated and the mononuclear cell pellet was stabilized in 1,200 µL of Ambion® RNAlater™ RNA Stabilization Reagent (Ambion Inc., Austin, Texas). RNAlater™ rapidly permeates tissue samples to stabilize and protect cellular RNA in situ thus rendering the samples suitable for cDNA synthesis, microarray and qPCR analyses. The BD CPT™ tube method was chosen to purposely exclude the neutrophils and reticulocytes as their presence may have obscured the genomic changes in the more active lymphocyte and monocyte populations (Connolly et al., 2004b).
4.3.6 Total RNA Isolation

After removing the RNAlater™ RNA Stabilization Reagent, 1,200 µL TRIzol® Reagent (Invitrogen, Life technologies, Karlsruhe, Germany) was added to each cell pellet sample. During sample homogenization, TRIzol® Reagent maintains total RNA integrity due to highly effective inhibition of RNase activity whilst disrupting cells and dissolving cellular components. Cells were lysed by repetitive pipetting (using a 20 gauge sterile needle and syringe) and incubated for 5 min at ambient temperature to permit complete dissociation of nucleoprotein complexes. Following homogenization, 240 µL of chloroform (0.2 mL of chloroform per mL of TRIzol® Reagents) was added to each sample and the tubes were vortexed for 15 sec and incubated for 3 min at room temperature. The samples were then centrifuged at 13,200 RPM for 10 min at 4°C to separate the homogenates into a clear upper aqueous phase (exclusively containing total RNA), an interphase, and a red lower organic phase (containing DNA and proteins). The aqueous phase of each sample was transferred to a fresh tube. An equal volume of 70% ethanol was added and the lysates were mixed well by pipetting. 700 µL of the sample of each sample was transferred to an individual RNeasy® mini spin column (Qiagen RNeasy® Mini Kit) which was placed in a 2 mL collection tube. The mini columns were centrifuged at 10,000 RPM for 15 sec at 20°C and the flow through was discarded. All subsequent centrifugation steps were also performed at 20°C to prevent the formation of precipitates which can clog the RNeasy® silica membrane. If the initial sample exceeded 700 µL, successive aliquots were centrifuged in the same RNeasy® mini spin column. Following the total RNA binding step, 700µL of Buffer RW1 (Qiagen RNeasy® Mini Kit) was added to the mini columns prior to centrifugation at 10,000 RPM for 15 sec. The subsequent flow through was discarded. 500 µL of Buffer RPE (Qiagen RNeasy® Mini Kit) was then added to the mini columns and the 15 sec centrifugation step was repeated. The Buffer RPE wash step was repeated with a subsequent 2 min centrifugation at 10,000 RPM. The spin columns were then placed in a new 2 mL collection tubes and centrifuged for 1 min at 10,000 RPM to ensure that no residual ethanol was carried over during the subsequent RNA elution step. The spin columns were then placed in a new 1.5 mL collection tube and 15 µL of RNase-free water (Qiagen RNeasy® Mini Kit) was added directly to each spin column membrane and allowed to incubate for 1 min. The mini columns were then centrifuged for 1 min at 10,000 RPM to elute the total RNA. The extracted total RNA was aliquoted and stored at -80°C until further analysis.
Total RNA Quantity and Quality Checks

For each sample, 1 µl of non-DNase treated total RNA was diluted (1:10 vol/vol with RNase-free water) for quantification of total RNA quantity and quality. The concentration and purity of total RNA samples was determined using a NanoDrop 1000 spectrophotometer (ND-1000, Thermo Fisher Scientific, Wilmington DE, USA). The absorbance of the diluted sample was determined at 230, 260 and 280 nm and the resulting 260/280 and 260/230 absorbance ratios were calculated automatically (refer to Appendix H). The concentration of total RNA (ng/µl) is based on the absorbance at 260 nm and is derived from a modified version of the Beer-Lambert equation (Beer’s Law).

\[
C = \frac{(A \times e)}{b}
\]

\(C\) = nucleic acid concentration (ng/µl)

\(A\) = absorbance (AU)

\(e\) = wavelength-dependent extinction coefficient (ng-cm/µl)

\(b\) = path length (cm)

The 260/280 absorbance ratio is used to assess the purity of total RNA; a ratio of ~2.0 is generally accepted as “pure” for total RNA. A ratio that is appreciably lower than 2.0 may indicate the unwanted presence of protein, phenol or other contaminants that absorb close to 280 nm. In the current investigation, all samples used for further analysis were deemed to be of acceptable purity with a mean (± SD) 260/280 ratio of 1.91 ± 0.11.

The quality of the total RNA was determined on an Agilent 2100 Bioanalyzer® (Agilent Technologies, Palo Alto, CA) and samples were run on RNA 6000 Nano LabChips. The Bioanalyzer software automatically calculates the ratio of the 18S to 28S ribosomal subunits and generates an RNA integrity number (RIN) with 1 being the most degraded and 10 being the most intact. In the current investigation, all samples used for further analysis were deemed to be of acceptable purity with a mean (± SD) RIN of 8.78 ± 2.63 (refer to Appendix H).
4.3.7 cDNA Microarray Analysis

To identify exercise-responsive genes, cDNA microarray analyses were performed on pre-exercise and 3 h post-exercise PBMC samples obtained from the four highest-ranked participants (based upon Sinclair coefficient scores – refer to Section 2.5.1.3) at the end of the 2 wk overload. Messenger RNA samples were submitted to the University of Auckland’s Centre for Genomics and Proteomics for analysis using the Human GeneChip® Gene 1.0 ST Arrays (Affymetrix, Santa Clara, CA, USA). The GeneChip® Gene 1.0 ST Array is designed to quantify the gene expression of well-annotated genes, using a single probe set per gene comprised of multiple probes along the entire length of the genomic locus (Pradervand et al., 2008). The content of the array was based upon the March 2006 (UCSC hg18, NCBI Build 36) human genome sequence assembly. For optimal sensitivity, the 100 ng Total RNA Labeling protocol was performed according to the GeneChip® Whole Transcript Sense Target Labeling Assay Manual (P/N 701880, Version 4). The GeneChip® Arrays were scanned on a GeneChip® Scanner 3000 7G (controlled by Affymetrix Command Console Software). The microarray analyses were performed in compliance with the “minimum information about a microarray experiment” (MIAME) standards (Brazma et al., 2001) with the intention of submitting the final data sets to the Gene Ontology database (Harris et al., 2004).

Normalisation Calculations and Quality Control Measures

Preliminary analysis of the microarray data was performed by a qualified technician at the Bioinformatics Institute at the University of Auckland. Normalisation and quality control measures were conducted as per the recommendations outlined by Heber et al. (2006) using Affymetrix Expression Console software (Affymetrix, Santa Clara, CA, USA) and R package aroma.affymetrix software (Bengtsson et al., 2008) (refer to Appendix H).

To identify outliers that can result from differences in amplification or labeling, raw array intensities box plots, raw log_2 probe hybridization intensities density plots and a Normalised Unscaled Standard Error (NUSE) plot were produced prior to performing a Robust Multiarray Average (RMA) algorithm correction (Dudoit et al., 2003; Irizarry et al., 2003). RMA value estimates are based upon a robust mean of the background-corrected perfect match intensities, and normalization was done with the use of quantile normalization strategies.
(Bolstad et al., 2003; Irizarry et al., 2003). Furthermore, Relative Log Expression (RLE) plots were created to determine if any array should be removed from subsequent analyses due to the presence of artefacts that could not be sufficiently removed following RMA (Heber et al., 2006) (refer to Appendix H).

**Candidate Gene Selection and Ontological Categorization**

The following selection criteria was used to select exercise-responsive genes for further analysis (using qPCR); 1) probe sets must exhibit a mean log-fold change of $\geq 1.4$ (Bouwens et al., 2007; Shi et al., 2006) between pre-exercise and 3 h post-exercise time points, 2) differentially expressed genes were deemed to be of interest if they had possible links with signalling pathways involved with skeletal muscle function and/or adaptation.

Pathway analysis was conducted using the Ingenuity Pathway Analysis software (Ingenuity Systems, Redwood City, CA, USA) which identifies significant networks, top functions and canonical pathways associated with the differentially expressed genes (Jiménez-Marín et al., 2009). The “Core Analysis” function was used to interpret the data in the context of biological processes, signalling pathways and associated networks (Jiménez-Marín et al., 2009). Biofunctions were grouped in: Diseases and Disorders; Molecular and Cellular Function; and, Physiological System Development and Function. The IPA software compiles network information from the Ingenuity Pathways Knowledge Base which is an extensive, manually curated database of functional interactions based upon peer-reviewed publications (Ingenuity Systems, Redwood City, CA, USA).
4.3.8 Real-Time Quantitative Polymerase Chain Reaction (qPCR)

**DNase Treatment**

Of the genes that met the selection criteria, Chemokine (C-C motif) Ligand 4 (CCL4), Chemokine (C-X-C motif) Receptor 4 (CXCR4) and DNA-Damage-Inducible Transcript 4 (DDIT4), were selected for further analysis (via qPCR) at five time points during the 4 wk training period.

Reverse transcription (RT) is the synthesis of a complementary template strand of DNA (cDNA) from total RNA, using the enzyme reverse transcriptase. Prior to performing the RT reactions, each RNA sample was purified from any contaminating DNA via the Ambion DNA-free™ Kit protocol (Ambion, Applied Biosystems). DNase treatment was considered necessary as two of the target gene assays of interest (DDIT4 assay ID: Hs00430304_g1 and CXCR4 assay ID: Hs00607978_s1) contained primers and probes designed with a single exon (i.e. does not cross an intro/exon boundary) and would therefore detect any contaminating DNA if present. Thus, contaminating DNA was digested to levels below the limit of qPCR detection by the addition of 0.1 volume of optimized DNase reaction buffer and 1 µl of recombinant DNase I (rDNase I). Samples were then incubated at 37°C for 25 min. Following the incubation, 0.1 volume of DNase Inactivation Reagent was added to each sample to remove the DNase I enzyme along with divalent cations which degrade RNA at temperatures above 60°C. Samples were incubated at room temperature for 2 min and were then centrifuged at 10,000 g for 1.5 min. The resulting supernatant which contained purified RNA was then transferred to a new tube.

**Reverse Transcription**

Total RNA was reverse transcribed using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen Life Technologies) according to the manufacturer’s instructions and recommendations. Two micrograms of RNA (for each sample) was reverse transcribed into cDNA in 20 µl reactions. Each reaction contained 4 µl of 5X VILO™ Reaction Mix (comprised of random primers, MgCl2, and dNTPs in an optimized qPCR buffer), 2 µl of 10X SuperScript® Enzyme Mix (comprised of SuperScript III Reverse Transcriptase,
RNaseOUT™ Recombinant Ribonuclease Inhibitor, and a proprietary helper protein) and the remaining 14 µl was comprised of RNA and DEPC-treated water (Invitrogen Life Technologies). The volume of RNA to DEPC-treated water was adjusted accordingly to achieve the same amount of total RNA per reaction (2.0 µg) to produce a final cDNA concentration of 100 ng/µl. Negative reverse transcriptase (RT) control reactions were also performed for each sample in which the 10X SuperScript® Enzyme Mix was omitted from the 20 µl reaction. The purpose of the RT control was to determine if any genomic DNA contamination was present following the DNase treatment steps. The reverse transcription procedure was performed in a Gene Amp PCR 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) using the following thermal cycle incubation parameters: 25°C for 10 min, 42°C for 120 min and 85°C for 5 min to terminate the reaction. Samples were chilled on ice and 10 µl of each cDNA sample was diluted (1:10 vol/vol with nuclease-free water) and divided into smaller aliquots which were stored at -20°C until further analysis.

**Real-Time Quantitative Polymerase Chain Reaction (qPCR) Protocol**

Real-time qPCR is the commonly used method of quantifying mRNA expression levels from a number of tissue types using fluorogenic probes. The TaqMan® probe assays takes advantage of the 5′ → 3′ exonuclease activity of Thermus aquaticus DNA polymerase (Heid et al., 1996; Holland et al., 1991) and enables the detection of low-fold transcriptional changes when this technique is performed correctly. The qPCR protocol used in the current investigation was adapted from commonly used protocols reported in the literature that have quantified the same genes of interest (Fruehauf et al., 2006; Koczan et al., 2008; Vincent et al., 2010). Predesigned sequence specific primer/probe sets were supplied as TaqMan® Gene Expression Assays (Assays on Demand, Applied Biosystems) containing a 20 × mix of unlabeled forward and reverse primers together with FAM-dye labeled TaqMan® MGB probe. The TaqMan® Gene Expression Assays used in the current investigation were Chemokine (C-C motif) Ligand 4 (CCL4) (Hs00237011_m1), Chemokine (C-X-C motif) Receptor 4 (CXCR4) (Hs00607978_s1), DNA-Damage-Inducible Transcript 4 (DDIT4) (Hs00430304_g1), Ribosomal protein, large P0 (RPLPO) (Hs99999902_m1) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (Hs99999905_m1). RPLPO and GAPDH were chosen as the reference genes for this investigation as they have been validated experimentally for particular use with peripheral blood mononuclear cells (Dheda et al., 2004) during exercise conditions (Büttner et al., 2007; Werner et al., 2009).
The 2 × TaqMan® Universal Mastermix II with AmpErase UNG, containing ROX as the passive reference dye (Applied Biosystems, PN 4428173), was used for each qPCR experiment according to the manufacturer’s recommendations. This particular Mastermix was chosen as UNG treatment can prevent the reamplification of carryover PCR contamination as it will degrade any PCR products containing dUTPs. For each 20 µl singleplex reaction, 2 µl of diluted cDNA (20 ng), 7 µl of RNase-free water, 1 µl of 20 × TaqMan® gene expression assay mix, and 10 µl of 2 × TaqMan® Universal Mastermix II (with AmpErase UNG) was used. For each participant, all samples were analyzed simultaneously (target genes and reference genes) in triplicate using an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). As a general control for unwanted nucleic acid contamination, no template controls (NTC) were run on each plate. Finally, common samples (inter-run calibrators) were included across all plates to allow the calculation of correction factors to remove the technical run-to-run variations between samples (Bustin et al., 2009; Derveaux et al., 2010; Hellemans et al., 2007).

The following thermal cycling conditions were adhered to:

<table>
<thead>
<tr>
<th></th>
<th>UNG Incubation</th>
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<th>PCR - 40 Cycles</th>
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<tr>
<td><strong>Temperature</strong></td>
<td>50</td>
<td>95</td>
<td>Denature 95</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>2:00</td>
<td>10:00</td>
<td>Anneal/Extend 60</td>
</tr>
</tbody>
</table>

**Normalisation Calculations and Quality Control Measures**

Prior to normalisation, the raw data amplification plots for each gene were inspected to ensure the baseline was set two cycles earlier than the cycle threshold (C_t) value for the most abundant sample. Furthermore, the corresponding threshold was examined to ensure that it fell within the exponential phase of the associated amplification plot (refer to Appendix H). The qPCR C_t data was exported into the qBase^PLUS_ software analysis program (Biogazelle, NV, Belgium) to determine the transcriptional fold changes in mRNA. The normalisation factors for each sample were monitored to account for variations in the amount of RNA, the efficiency of reverse transcription and the stability of the reference genes. The mean (± SD)
of the normalisation factors across all experimental samples and qPCR runs was 1.19 ± 0.63 indicating a low variability between samples (Hellemans et al., 2007). Variation of between 2-3-fold is generally acceptable for qPCR analyses due to experimental variation which is accounted for during the subsequent normalisation steps (D’Haene et al., 2010; Hellemans et al., 2007; Vandesompele et al., 2002a).

The final fold changes for mRNA expression levels pre- and 3 h post-exercise (during overload and recovery) were determined from the corresponding C_t values using $2^{-\Delta\Delta C_t}$. Using this method, the target gene expression was normalized to the baseline sample (assigned the arbitrary value of 1.0) and the geometric mean of the reference genes (Vandesompele et al., 2002b) using the following equations (Livak et al., 2001; Schmittgen et al., 2008):

Equation 1: $\Delta C_t = C_t$ mean target gene $- C_t$ geometric mean reference genes

Equation 2: $\Delta \Delta C_t = \Delta C_t$ sample time point $- \Delta C_t$ sample baseline

Equation 3: $R = 2^{\Delta\Delta C_t}$

*Sample time points = pre- and post-exercise during overload and recovery training periods.

The comparative C_t method assumes that all targets and reference genes amplify with a PCR efficiency close to 1 and presents the data as a ‘fold-change’ in expression (Livak et al., 2001; Schmittgen et al., 2008). According to Applied Biosystems (TaqMan® Application Note), the TaqMan® Gene Expression Assays amplify with an efficiency of 100 ± 10%.

The experimental variation of C_t replicates was assessed in qBasePLUS using the quality control criteria of ≤0.5 cycles between replicates for each sample/gene pairing (D’Haene et al., 2010). Replicates that exceeded the quality control criteria were excluded from further analyses. Across all plates, ≤9% of replicates were excluded from subsequent analyses. The maximum variation between the remaining replicates were <0.3 cycles.
4.3.9 Macrophage Inflammatory Protein-1β Analysis

In the context of the current investigation, the translated protein encoded by the gene CCL4 will be referred to as Macrophage Inflammatory Protein-1β (MIP-1β) (Bacon et al., 2002).

It is acknowledged that mRNA levels are not effective at predicting the abundance and activity of the corresponding protein product (Bustin, 2002; 2009; Hittel et al., 2007; Yi et al., 2008). Therefore, in light of the cDNA microarray results, circulating MIP-1β levels were measured in pre-exercise, immediately post-exercise and 3 h post-exercise plasma samples obtained at the end of the 2 wk overload and 1 wk recovery period using a commercially available ELISA kit (QuantiKine, R&D Systems, Minneapolis, MN, USA). The manufacturer’s stated sensitivity of the kit was 11.0 pg/mL with an intra-assay coefficient of variation of 3.6%. The required reagents were made from the components supplied within the kit (refer to Appendices G) and the manufacturer’s standard procedures for serum/plasma samples were followed.

**ELISA Procedure**

Prior to use, all reagents were brought to room temperature and plasma samples were thawed only once before analysis. 100 µL of Assay Diluent RD1X was added to each well. All samples and standards were analyzed in duplicate and 150 µL of each sample and standard were loaded into each of the assigned ELISA-plate wells. The plate was then covered with the adhesive strip and was left to incubate for 2 h at room temperature. Following the incubation, the plate was washed three times with Wash Buffer. Following the plate wash, 200 µL of MIP-1β Conjugate was added to each well. The plate was then covered with a new adhesive strip and was left to incubate for 2 h at room temperature. The plate wash step was then repeated after which 200 µL of Substrate Solution was added to each well. The plate was left to incubate in the dark for 20 min at room temperature. 50 µL of Stop Solution was added to each well and the resulting absorbances were read at 450 nm and 562 nm for wavelength correction using an ELx808 Ultra Microplate Reader (Bio-Tek Instruments, Swedesboro, NJ, USA).
**Colorimetric Analysis**

The known MIP-1β concentrations of the standards were then plotted against their respective absorbances and the resultant regression line had an $R^2$ value of 0.9907. The MIP-1β concentration of each of the samples was then calculated using the regression equation obtained from the standard curve.

**4.3.10 Protein Carbonyl Analysis**

To determine the protein oxidative response to variations in training load, plasma protein carbonyl (PC) levels were measured pre-exercise, immediately post-exercise and 3 h post-exercise at the end of the 2 wk overload and 1 wk recovery period. Plasma PC levels have previously been used as an early indicator of skeletal muscle damage (Bloomer et al., 2005a; Dalle-Donne et al., 2003a; Lee et al., 2002). Plasma PC levels were analyzed using a commercially available ELISA kit (BioCell Corporation Ltd, Auckland, New Zealand). The manufacturer’s stated sensitivity of the kit was 0.06 nmol/mg with an intra-assay coefficient of variation of 5%. The required regents were made from the components supplied within the kit (refer to Appendices G) and the manufacturer’s standard procedures for samples containing 0.4-80 mg/ml of protein were followed accordingly.

**Sample Derivatisation with Dinitrophenylhydrazine (DNP)**

Prior to use, all reagents were brought to room temperature and plasma samples were thawed only once before analysis. 25 µL of deionized water was then added to each of the 6 oxidized protein standards and the carbonyl control and they were incubated for 2 h at 37°C. During this time, 200 µL of *diluted DNP solution* was added to forty eight 1.5 mL reaction tubes. A duplicate set of tubes containing 1 mL of EIA buffer was then prepared. 5 µL of each standard, control or sample was then added to the appropriate reaction tube and incubated for 45 min at room temperature. 5 µL of the derivatised sample was then added to the appropriate duplicate tube containing 1mL of EIA buffer.
**ELISA Procedure**

All samples and standards were analyzed in duplicate and 200 µL of each sample and standard (in EIA buffer) were loaded into each of the assigned ELISA-plate wells. The plate was then covered with the adhesive strip and was left to incubate overnight at 4 °C. Following the overnight incubation, the plate was washed five times with EIA buffer and this wash step was repeated in the same manner between each of the following steps: blocking the wells with 250 µL of *diluted blocking solution* for 30 min at room temperature; addition of 200 µL/well of diluted anti-DNP-biotin-antibody and incubation for 1 hour at 37 °C; addition of 200 µL/well of *diluted streptavidin-HRP* and incubation for 1 hour at room temperature.

**Colorimetric Analysis**

200 µL of chromatin reagent was added to each of the plate wells and the color was allowed to develop for 20 min at room temperature. The reaction was followed at 630 nm and was stopped with the addition of 100 µL/well of stopping reagent. The plate was gently shaken and the resulting absorbances were then read at 450 nm using an ELx808 Ultra Microplate Reader (Bio-Tek Instruments, Swedesboro, NJ, USA). The known protein carbonyl concentrations of the oxidized protein standards were then plotted against their respective absorbances. The resultant regression line had an $R^2$ value of 0.9632. The carbonyl content of each of the samples was then calculated using Equation A (see below) and the regression factors obtained from the standard curve.

**Regression Line Equation:**

$$y = a \cdot \exp(c \alpha)$$

$y$ = absorbance; $a$ = gradient; $c$ = exponent of gradient; $\alpha$ = protein carbonyl concentration.

**Equation A:**

$$\alpha = \left( \ln \left( \frac{y}{a} \right) \right) \frac{1}{c}$$
4.3.11 Psychological Assessment

The Profile of Mood States (POMS) (McNair et al., 1971) and the Daily Analysis of Life Demands of Athletes (DALDA) (Rushall, 1990) questionnaires were chosen to quantify the affective responses to variations in training stress due to their wide use in sport and exercise science research (Beedie et al., 2000; Berger et al., 2000; Coutts et al., 2007; Halson et al., 2002; Hooper et al., 1997; Lambert et al., 2006; LeUnes et al., 1998; Morgan et al., 1988; Nicholls et al., 2009; Snow et al., 1994; Terry et al., 2000).

Every day for the duration of the baseline, overload and recovery training periods, all participants completed the DALDA questionnaire (Rushall, 1990) (refer to Appendix C). The DALDA is divided into Parts A and B, which represent the general stress sources that occur in the everyday life of an athlete and the resulting signs/symptoms, respectively (Rushall, 1990). All participants had previous experience with using the DALDA questionnaire in training and were required to complete the questionnaire at the same time each day prior to the afternoon training session.

During the 2 week overload and 1 week recovery period, participants also completed the 65-item POMS questionnaire (McNair et al., 1971) (refer to Appendix C). The POMS questionnaire provides a measure of the tension/anxiety, depression, anger, vigor, fatigue and confusion levels of the participant. Participants were required to complete the POMS based on how they felt “right now” at the same time each day prior to the morning training session. Each mood item was rated on a 5-point scale as follows: 0 = “not at all”, 1 = “a little”, 2 = “moderately”, 3 = “quite a bit”, 4 = “extremely”. A mean weekly group score was calculated for each mood state by summing the responses obtained from each participant for the adjectives that defined the mood state. All mood state items were positively weighted with two exceptions receiving negative weightings; “relaxed” in the Tension/Anxiety scale and “efficient” in the Confusion scale (McNair et al., 1971). A Total Mood Disturbance Score (TMDS) was calculated by adding the weekly means of the five negative mood states together and subtracting the positive mood state, vigor (Tension/Anxiety + depression + anger + fatigue + confusion – vigor) (Morgan et al., 1988).
Each week, the lead investigator personally issued and collected the DALDA and POMS questionnaires as previous research has shown that a single booklet format results in high non-completion rates (Nicholls et al., 2006; 2009).

4.3.12 Data Presentation and Statistics

Data were analyzed with SigmaPlot 11.0 statistical software (Chicago, IL). Data are presented as mean ± SD with the statistical significance set at $P < 0.05$. Where applicable, effect sizes were calculated according to the method of Cohen where $d = 0.8$ is considered a large effect, $d = 0.5$ is moderate, and $d = 0.2$ a small effect size (Cohen, 1992). Statistical procedures specific to each variable are described below:

Participants

Independent samples $t$-tests were used to assess differences between the descriptive characteristics of the participants.

Training Program and Performance

A series of one way repeated-measures ANOVA were used to investigate 1) each weekly program variable (Total Load, Total Repetitions, Mean Training Load) and 2) changes in performance. Where a significant difference between variables was determined by ANOVA, post-hoc paired comparisons were made using the method of Student-Newman-Keuls. Effect sizes were calculated according to the method of Cohen (Cohen, 1992). Finally, Pearson product moment correlations were used to test for an association between vertical jump estimated peak power (W) and weightlifting performance (snatch and C&J).

Psychological Questionnaires

For the DALDA analysis, a series of two factor response (A, B, C) by week (Baseline, Week 2 – Overload, Week 3 – Overload, Week 4 - Recovery) repeated-measures ANOVA were used to investigate the changes in the signs (Part A) and symptoms (Part B) of stress. The
analyses tested for main effects of response and week and any interaction between response and week.

For the analysis of the POMS data, a two factor week (Baseline, Week 2 – Overload, Week 3 – Overload, Week 4 - Recovery) by mood state (tension/anxiety, depression, anger, vigor, fatigue and confusion) repeated-measures ANOVA tested for main effects of week and mood state and any interaction between week and mood state. Where a significant difference between variables was determined by ANOVA for DALDA and POMS scores, post-hoc paired comparisons were made using the method of Student-Newman-Keuls. To determine the influence of the training week on the weekly Total Mood Disturbance Scores, a one-way ANOVA was performed.

To test for an association between weekly POMS scores (individual mood state scores and Total Mood Disturbance Scores), training load and performance (maximal vertical jump, snatch and clean and jerk), Pearson product moment correlations were used to evaluate the relative (percentage) change in POMS scores and the relative (percentage) change in training load and performance between each week (i.e. Week 2-3 and Week 3-4) and relative to Baseline scores (i.e. Baseline – Week 2, Baseline – Week 3 and Baseline – Week 4).

**mRNA Quantification**

Linear modeling was performed on the microarray data using the BioConductor Limma software package (BioConductor: Open Source Software for Bioinformatics). A moderated paired t-test was performed, according to the method of Smyth (2005), on complete pre-exercise and 3 h post-exercise array pairs (an array pair was excluded from the statistical analysis due to poor hybridization). A Benjamini-Hochberg correction for multiple testing was used to control the false-discovery rate at 0.05 (Benjamini et al., 1995).

For the qPCR data, a two factor condition (overload, recovery) by time (pre-exercise, post-exercise) repeated-measures ANOVA was used to investigate the final fold changes for mRNA expression levels. The analyses tested for main effects of condition and time and any interaction between condition and time.
Protein Carbonyl and MIP-1β Assays

Two factor condition (overload, recovery) by time (pre-exercise, 0 min post-exercise and 3 h post-exercise) repeated-measures ANOVAs were used to investigate the changes in plasma protein carbonyl and MIP-1β levels. The analyses tested for main effects of condition and time and any interaction between condition and time. Where a significant difference between variables was determined by ANOVA, post-hoc paired comparisons were made using the method of Student-Newman-Keuls. Pearson product moment correlations were also used to evaluate the relative (percentage) change in plasma protein carbonyl and MIP-1β levels and the relative (percentage) change in training load and performance between Week 3 (overload) and Week 4 (recovery).
4.4 Results

4.4.1 Participants

The mean age, body mass and number of training years did not differ between males and females. In addition, no significant changes in participant body mass occurred during the study period. However, male weightlifters were taller ($P < 0.05; d = 1.4$) and had a greater snatch ($P < 0.05; d = 1.6$) and clean and jerk ($P < 0.05; d = 1.6$) than female weightlifters (Table 4-2).

Table 4-2: Physical and performance characteristics of the competitive weightlifters at baseline. Data are presented as mean (± SD). *Significant difference between groups ($P < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>All ($N = 7$)</th>
<th>Men ($N = 4$)</th>
<th>Women ($N = 3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>22.9 ± 4.3</td>
<td>21.5 ± 3.7</td>
<td>26.7 ± 5.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.4 ± 9.1</td>
<td>171.0 ± 4.3*</td>
<td>158.0 ± 8.5</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>80.4 ± 18.6</td>
<td>82.6 ± 16.9</td>
<td>77.5 ± 24.2</td>
</tr>
<tr>
<td>Vertical Jump (cm)</td>
<td>63.0 ± 15.0</td>
<td>73.5 ± 4.0*</td>
<td>48.3 ± 9.1</td>
</tr>
<tr>
<td>Vertical Jump Peak Power (W)</td>
<td>8600.0 ± 1078.0</td>
<td>9345.8 ± 480.3*</td>
<td>7605.0 ± 735.2</td>
</tr>
<tr>
<td>Snatch (kg)</td>
<td>107.4 ± 32.2</td>
<td>129.3 ± 21.2*</td>
<td>78.8 ± 14.3</td>
</tr>
<tr>
<td>Clean &amp; Jerk (kg)</td>
<td>137.3 ± 41.1</td>
<td>165.5 ± 25.5*</td>
<td>99.7 ± 19.1</td>
</tr>
<tr>
<td>Training Years</td>
<td>7.1 ± 3.0</td>
<td>7.3 ± 3.6</td>
<td>7.0 ± 2.7</td>
</tr>
</tbody>
</table>

4.4.2 Training Program

A main effect of week existed for the Total Weekly Load (kg), the Total Number of Repetitions, and the Mean Weekly Training Load (kg) ($P < 0.001$). Post-hoc paired comparisons showed that the Total Weekly Training Load (kg) was greater during Week 2, 3 and 4, when compared to the Baseline week ($P < 0.05; d = 1.0 – 1.4$) (Figure 4-4). In addition, a greater number of repetitions were performed during Week 2, 3 and 4, when compared to the Baseline week ($P < 0.001; d = 1.8-1.9$). Furthermore, the number of
repetitions performed during Week 2 and 3 (overload) were greater \( (P < 0.001; d = 1.7) \) than the number performed during Week 4 (recovery). During Week 2 and 3 (overload), the Mean Weekly Training Load (kg) was increased when compared to the Baseline week \( (P < 0.001; d = 0.4 - 0.5) \). Finally, the Mean Weekly Training Load (kg) of Week 2 and 3 (overload) was greater \( (P < 0.05; d = 0.2 - 0.3) \) than that of Week 4 (recovery).

**Figure 4-4:** Variations in the total weekly training load (kg) during the four week training period. Data are presented as mean (± SD). *Significant difference from Week 1 - Baseline \( (P < 0.05) \); ‡Significant difference from Week 4 - Recovery \( (P < 0.05) \).

### 4.4.3 Performance Testing

**Vertical Jump Performance**

There was a main effect of week \( (P < 0.05) \) for vertical jump scores (cm) and mean vertical jump peak power (W). The mean vertical jump scores (cm) in Week 2 (overload) and Week 3 (overload) were lower than that of Baseline by \( 5.9 \pm 5.4\% \) and \( 7.2 \pm 6.5\% \) \( (P < 0.05; d = 0.3) \), respectively. In addition, mean peak power (W) during Week 2 (overload) and Week 3
(overload) were also lower than that of Baseline ($P < 0.05$; $d = 0.2-0.3$). No significant difference in vertical jump performance or estimated peak power outputs between Week 4 (recovery) and Baseline was evident, indicating a restoration in performance (Table 4-3).

**Snatch and Clean and Jerk Performance**

There was a main effect of training week for snatch performance only ($P < 0.05$). Post-hoc paired comparisons demonstrated that the mean snatch performance in Week 3 (overload) was lower than that of the current personal best levels by $4.3 \pm 3.7\%$ ($P < 0.001$; $d = 0.1$) (Table 4-3). No significant difference in snatch performance between Week 4 (recovery) and Baseline was evident, indicating a restoration in performance. Finally, estimated peak power (W) was strongly correlated with snatch and C&J performance across all time points ($P < 0.001$; $R^2 = 0.9$).

**Table 4-3: Performance testing results across the 2 week overload and 1 week recovery training periods in relation to baseline/personal best values. Data are presented as mean (± SD). *Significant difference from baseline/personal best ($P < 0.05$).**

<table>
<thead>
<tr>
<th>Performance Variable</th>
<th>Baseline/Personal Best</th>
<th>Week 2 - Overload</th>
<th>Week 3 - Overload</th>
<th>Week 4 - Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snatch (kg)</td>
<td>103.6 ± 31.9</td>
<td>100.3 ± 28.7</td>
<td>99.1 ± 30.6*</td>
<td>102.3 ± 31.0</td>
</tr>
<tr>
<td>Difference from Personal Best (%)</td>
<td>-2.7 ± 4.8</td>
<td>-4.3 ± 3.7*</td>
<td>-1.1 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Clean &amp; Jerk (kg)</td>
<td>133.6 ± 40.9</td>
<td>127.9 ± 39.5</td>
<td>128.4 ± 36.2</td>
<td>131.9 ± 40.1</td>
</tr>
<tr>
<td>Difference from Personal Best (%)</td>
<td>-3.9 ± 6.0</td>
<td>-3.0 ± 4.4</td>
<td>-1.1 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Vertical Jump (cm)</td>
<td>62.7 ± 14.7</td>
<td>59.0 ± 14.4*</td>
<td>58.4 ± 15.2*</td>
<td>60.4 ± 14.4</td>
</tr>
<tr>
<td>Difference from Baseline (%)</td>
<td>-5.9 ± 5.4*</td>
<td>-7.2 ± 6.5*</td>
<td>-3.7 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Vertical Jump Peak Power (W)</td>
<td>8599.8 ± 1077.6</td>
<td>8373.0 ± 1055.2*</td>
<td>8340.7 ± 1027.3*</td>
<td>8464.8 ± 993.4</td>
</tr>
</tbody>
</table>
4.4.4 cDNA Microarray

The microarray analysis was used to identify interesting and robust gene changes following HIRE in competitive weightlifters. Using a conservative false discovery rate of $P < 0.05$ and a log-fold change of $\geq 1.4$ (Bouwens et al., 2007), 202 differentially expressed genes were identified for ontological categorization. The top canonical pathways (identified by Ingenuity Pathway Analysis) from the genes differentially expressed from pre-exercise to 3 h post-exercise are presented in Table 4-4. Exercise-responsive genes were most frequently associated with the following networks: 1) Cell-To-Cell Signalling and Interaction, Hematological System Development and Function, Immune Cell Trafficking; 2) Organismal Survival, Inflammatory Response, Cell-To-Cell Signalling and Interaction; 3) Cell Cycle, Cell Death, Cancer (refer to Table 4-5).

Based upon our candidate gene selection criteria (refer Section 4.3.7), the following genes were selected for qPCR analyses at baseline and pre- and 3 h post-exercise after overload and recovery training; Chemokine Ligand 4 (CCL4) (log-fold change of 1.45), Chemokine Receptor 4 (CXCR4) (log-fold change of 1.44) and DNA-Damage-Inducible Transcript 4 (DDIT4) (log-fold change of 1.64) (refer to Table 4-5).
Table 4-4: Top canonical pathways and biological functions (identified by Ingenuity Pathway Analysis) of the genes differentially expressed between pre-exercise and 3 hour post-exercise blood samples ($P < 0.05$, log-fold change of $\geq 1.4$). Data are presented as mean ($\pm$ SD).

<table>
<thead>
<tr>
<th>Canonical Pathway</th>
<th>$P$ Value</th>
<th>Number of Differentially Expressed Molecules Relative to Total In Pathway (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen Presentation Pathway</td>
<td>$5.0 \times 10^{-7}$</td>
<td>5/43 (11.6)</td>
</tr>
<tr>
<td>Allograft Rejection Signalling</td>
<td>$7.7 \times 10^{-7}$</td>
<td>5/59 (8.5)</td>
</tr>
<tr>
<td>Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells</td>
<td>$1.3 \times 10^{-6}$</td>
<td>5/52 (9.6)</td>
</tr>
<tr>
<td>OX40 Signalling Pathway</td>
<td>$2.3 \times 10^{-6}$</td>
<td>5/61 (8.2)</td>
</tr>
<tr>
<td>Communication between Innate and Adaptive Immune Cells</td>
<td>$1.8 \times 10^{-5}$</td>
<td>5/93 (5.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biological Functions</th>
<th>$P$ Value</th>
<th>Number of Differentially Expressed Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diseases and Disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatological Diseases and Conditions</td>
<td>$\leq 4.2 \times 10^{-2}$</td>
<td>11</td>
</tr>
<tr>
<td>Immunological Disease</td>
<td>$\leq 4.1 \times 10^{-2}$</td>
<td>19</td>
</tr>
<tr>
<td>Inflammatory Disease</td>
<td>$\leq 4.1 \times 10^{-2}$</td>
<td>23</td>
</tr>
<tr>
<td>Inflammatory Response</td>
<td>$\leq 4.9 \times 10^{-2}$</td>
<td>12</td>
</tr>
<tr>
<td>Genetic Disorder</td>
<td>$\leq 3.4 \times 10^{-2}$</td>
<td>19</td>
</tr>
<tr>
<td><strong>Molecular and Cellular Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Synthesis</td>
<td>$\leq 1.1 \times 10^{-2}$</td>
<td>10</td>
</tr>
<tr>
<td>Cell Signalling</td>
<td>$\leq 4.6 \times 10^{-2}$</td>
<td>8</td>
</tr>
<tr>
<td>DNA Replication, Recombination, and Repair</td>
<td>$\leq 2.7 \times 10^{-2}$</td>
<td>4</td>
</tr>
<tr>
<td>Nucleic Acid Metabolism</td>
<td>$\leq 4.6 \times 10^{-2}$</td>
<td>5</td>
</tr>
<tr>
<td>Small Molecule Biochemistry</td>
<td>$\leq 4.6 \times 10^{-2}$</td>
<td>14</td>
</tr>
</tbody>
</table>
Table 4-4: Continued.

<table>
<thead>
<tr>
<th>Biological Functions</th>
<th>P Value</th>
<th>Number of Differentially Expressed Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological System Development and Function</td>
<td>≤4.9 x 10^{-2}</td>
<td>6</td>
</tr>
<tr>
<td>Cell-mediated Immune Response</td>
<td>≤3.6 x 10^{-2}</td>
<td>5</td>
</tr>
<tr>
<td>Immune Cell Trafficking</td>
<td>≤4.9 x 10^{-2}</td>
<td>3</td>
</tr>
<tr>
<td>Hematopoiesis</td>
<td>≤3.6 x 10^{-2}</td>
<td>4</td>
</tr>
<tr>
<td>Connective Tissue Development and Function</td>
<td>≤4.6 x 10^{-3}</td>
<td>1</td>
</tr>
</tbody>
</table>

Canonical pathways are known pathways for which there is a significant association with the identified molecules in each data set. Biological functions are composed of the molecules in each data set that are known to be involved in the various functions listed.

Table 4-5: The genes associated with various biological functions during the early recovery (i.e. 3 h post-exercise) from high-power, high-intensity resistance exercise in competitive weightlifters.

<table>
<thead>
<tr>
<th>Top Functions</th>
<th>Associated Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-To-Cell Signalling and Interaction, Hematological System Development and Function, Immune Cell Trafficking</td>
<td>ADA, BCL11B, C3AR1, CCL3, <strong>CCL4</strong>, CD80, CFB, CLEC4E, CPT1A, <strong>CXCR4</strong>, DDR2, DPP4, EBI3, EIF4B, GSTM2, HLA-B, HLA-DPB1, HLA-DRA, HLA-E, IFNG, IgG, IL13, IL7R, INS, MAGEA3/MAGEA6, MAOA, NLRC5, OAS2, PGF, RFX5, TNF, TSC22D3, U1 snRNP, ULBP1, ULBP2, GSTM2, HLA-B, HLA-DPB1, HLA-DRA, HLA-E, IFNG, IgG, IL13</td>
</tr>
</tbody>
</table>
Table 4-5: Continued.

<table>
<thead>
<tr>
<th>Top Functions</th>
<th>Associated Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organismal Survival, Inflammatory Response, Cell-To-Cell Signalling and Interaction</td>
<td>TBC1D3F, NPIP, NR1H3, ORM1/ORM2, PF4, PGAM1, PGR, Ras homolog, SERPINE1, HGF, HSPA1A/HSPA1B, Ilk, IL6, IL7, IL27, IL32, IL17F, IL18RAP, IL1B, IL1R1, IL23A, KLK3, NANOG, APOA1, APOE, AR, C3, CEBPD, CFHR1, CSF1, DEFA1, FGF7, Fibrinogen, FKBP5, GPR125</td>
</tr>
<tr>
<td>Cell Cycle, Cell Death, Cancer</td>
<td>SMARCA2, STOM, TP53, TP63, TRIB3, TSG101, YY1 HIPK2, Hsp27, HSPA8, MAX, MDM2, NPM1, NR3C1, PML, PRKCB, Rnr, RPL5, RPS9, RPS27, RPS28, APOE, ATG4B, BAG6, CARM1, CREBBP, CUL5, DAXX, DDIT4, DHFR, DMAP1, EIF5A, EP300, ESR1, FAS</td>
</tr>
<tr>
<td>Cellular Assembly and Organization, Cell Morphology, Energy Production</td>
<td>LETM1, MRPL36</td>
</tr>
<tr>
<td>Lipid Metabolism, Small Molecule Biochemistry, Amino Acid Metabolism</td>
<td>AKR1C1/AKR1C2, TGFB1</td>
</tr>
<tr>
<td>Cancer, Cell Morphology, Cell-To-Cell Signalling and Interaction</td>
<td>MALAT1, NCAM1</td>
</tr>
<tr>
<td>Cellular Response to Therapeutics, Tissue Development, Cell Cycle</td>
<td>AURKA, AURKAIP1</td>
</tr>
<tr>
<td>Cancer, Gastrointestinal Disease, Gene Expression</td>
<td>BDP1, BRF1</td>
</tr>
<tr>
<td>Cardiovascular System Development and Function, Carbohydrate Metabolism, Small Molecule Biochemistry</td>
<td>ADCY, CALCB</td>
</tr>
<tr>
<td>Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance</td>
<td>OSBPL1A, RAB7A</td>
</tr>
<tr>
<td>Genetic Disorder, Neurological Disease, Cellular Assembly and Organization</td>
<td>CCDC53, FAM21A, KIAA0196, KIAA1033</td>
</tr>
</tbody>
</table>
4.4.5 Real-Time Quantitative Polymerase Chain Reaction (qPCR)

A main effect of experimental time point \((P < 0.05)\) existed for CXCR4 and DDIT4 mRNA expression \((P < 0.05)\). In addition, there was an interaction between training period and time point \((P < 0.05)\) for CCL4 and DDIT4 mRNA expression in PBMCs. Post-hoc paired comparisons showed a robust exercise-induced response in CCL4, CXCR4 and DDIT4 following the 2 wk overload \((P < 0.05; d = 0.7 – 1.1)\) when compared to the 1 wk recovery (Figure 4-5). No significant pre- to post-exercise effect was detected for these genes following the 1 wk recovery period.

![Figure 4-5: The changes in candidate gene expression from pre-exercise to 3 hours post-exercise at the end of the overload and recovery training periods. Pre- and post-exercise values were normalised to the baseline sample. Data are presented as mean (± SD). *Significant difference from pre-exercise \((P <0.05)\); ‡Significant difference for the same time point between training periods \((P <0.05)\).](chart.png)
4.4.6 Macrophage Inflammatory Protein-1β

There was a main effect of training period ($P < 0.05$) and an interaction between training period and time ($P < 0.001$) for plasma MIP-1β levels. Post-hoc paired comparisons demonstrated that MIP-1β levels decreased from pre- to 3 h post-exercise after the overload (-42.8 ± 35.7%) and recovery (-64.7 ± 5.2%) training periods ($P < 0.05; d = 0.6 - 1.1$) (Figure 4-6). No correlations existed between plasma MIP-1β levels, training loads and/or performance measures.

![Figure 4-6: Plasma Macrophage Inflammatory Protein-1β (MIP-1β) levels during the final training session at the end of the 2 week “overload” period (♦) and 1 week “recovery” (□) period. Data are presented as mean (± SD). *Significant difference from Pre-Exercise ($P < 0.05$); ‡Significant difference from Post-Exercise (3 h) ($P < 0.05$).](image-url)
4.4.7 Protein Carbonyl

A main effect of training period \((P < 0.05)\) and an interaction between training period and time \((P < 0.001)\) was evident for plasma protein carbonyl (PC) levels. Post-hoc paired comparisons demonstrated that during the 2 wk overload period, PC levels were greater pre- and immediately post-exercise (0 min) when compared to 3 h post-exercise \((P < 0.05; d = 1.3 - 1.4)\). Conversely, during the 1 wk recovery period, pre- and immediately post-exercise (0 min) PC levels were lower when compared to 3 h post-exercise \((P < 0.05; d = 1.1 - 1.2)\) (Figure 4-7). Furthermore, the 2 wk overload resulted in greater pre- \((35.2 \pm 23.2\%)\) and immediate post-exercise \((35.3 \pm 10.8\%)\) PC response, indicative of enhanced tissue protein oxidation, when compared with the 1 week recovery period \((P < 0.05; d = 1.5 - 1.6)\) (Figure 4-7). No correlations existed between plasma PC levels, training loads and/or performance measures.

Figure 4-7: Plasma protein carbonyl levels during the final training session at the end of the 2 week “Overload” period (♦) and 1 week “Recovery” (□) period. Data are presented as mean \((\pm SD)\). *Significant difference from Post-Exercise (3 h) \((P < 0.05)\); ‡Significant difference between periods at the same time point \((P < 0.05)\).
4.4.8 Psychological Assessment

DALDA Questionnaire: Part A (Sources of Stress)

There was a main effect of response \( (P < 0.001) \) in Part A (sources of stress) of the DALDA questionnaire. Post-hoc paired comparisons demonstrated that the weekly number of “B” (normal) responses were greater than the weekly number of “A” (worse than normal) and “C” (better than normal) responses across all weeks \( (P < 0.05; d = 1.7 - 1.9) \) (Table 4-6). Furthermore, participants reported a greater number of “A” (worse than normal) responses during the first week of overload (Week 2) when compared to the Baseline week \( (P < 0.05; d = 1.3) \) (refer to Figures 4-8, 4-9 and 4-10). The most common changes in the sources of stress were related to “Sleep” and “Diet” which demonstrated increased “A” (worse than normal) response rates during Week 2 (overload) and 3 (overload), relative to the Baseline week \( (P < 0.05) \) (Table 4-7).

Table 4-6: Total weekly “A” (worse than normal), “B” (normal) and “C” (better than normal) responses for Part A (Sources of Stress) of the Daily Analysis of Life Demands of Athletes questionnaire. Data are presented as mean (± SD). *Significant difference from “B” (normal) response within week \( (P < 0.05) \); ‡Significant difference in response from Baseline week \( (P < 0.05) \).

<table>
<thead>
<tr>
<th>Weekly Response</th>
<th>Baseline</th>
<th>Week 2 - Overload</th>
<th>Week 3 - Overload</th>
<th>Week 4 - Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.3 (4.2)*</td>
<td>13.3 (5.6)*‡</td>
<td>9.7 (4.3)*</td>
<td>10.1 (6.1)*</td>
</tr>
<tr>
<td>B</td>
<td>46.0 (4.2)</td>
<td>41.1 (8.4)</td>
<td>44.7 (9.9)</td>
<td>43.0 (12.2)</td>
</tr>
<tr>
<td>C</td>
<td>12.0 (2.6)*</td>
<td>8.6 (7.7)*</td>
<td>8.6 (8.5)*</td>
<td>9.9 (9.0)*</td>
</tr>
</tbody>
</table>
Figure 4-8: Changes in the Daily Analysis of Life Demands of Athletes Part A (sources of stress) - “A” (worse than normal) scores during baseline, two weeks of overload training (grey) and one week of recovery training.

Figure 4-9: Changes in the Daily Analysis of Life Demands of Athletes Part A (sources of stress) - “B” (normal) scores during baseline, two weeks of overload training (grey) and one week of recovery training.
Figure 4-10: Changes in the Daily Analysis of Life Demands of Athletes Part A (sources of stress) - “C” (better than normal) scores during baseline, two weeks of overload training (grey) and one week of recovery training.
Table 4-7: Total weekly “A” (worse than normal), “B” (normal) and “C” (better than normal) responses for each “Sources of Stress” from Part A of the Daily Analysis of Life Demands of Athletes questionnaire. Data are presented as mean (± SD). *Significant difference from Baseline (P < 0.05); §Significant difference from Week 2 – Overload (P < 0.05); ¥Significant difference from Week 4 – Recovery (P < 0.05).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Baseline</th>
<th>Week 2 - Overload</th>
<th>Week 3 - Overload</th>
<th>Week 4 - Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Diet</td>
<td>1.3 (1.0)</td>
<td>4.1 (0.7)</td>
<td>1.6 (1.5)</td>
<td>3.0 (1.9)*</td>
</tr>
<tr>
<td>Home-Life</td>
<td>0.9 (1.1)</td>
<td>6.1 (1.1)</td>
<td>0 (0)</td>
<td>0.6 (0.8)</td>
</tr>
<tr>
<td>School/Work</td>
<td>0.7 (1.0)</td>
<td>4.9 (1.1)</td>
<td>1.3 (1.3)</td>
<td>1.9 (1.6)</td>
</tr>
<tr>
<td>Friends</td>
<td>0.0 (0.0)</td>
<td>5.4 (1.4)</td>
<td>1.9 (1.5)</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td>Sport Training</td>
<td>0.1 (0.4)</td>
<td>4.4 (0.5)</td>
<td>2.6 (0.8)</td>
<td>1.3 (2.1)</td>
</tr>
<tr>
<td>Climate</td>
<td>0.1 (0.4)</td>
<td>6.0 (1.3)</td>
<td>0.9 (1.1)</td>
<td>0.6 (0.8)</td>
</tr>
<tr>
<td>Sleep</td>
<td>0.9 (0.9)¥</td>
<td>3.9 (0.7)</td>
<td>2.3 (1.1)</td>
<td>4.0 (0.6)*</td>
</tr>
<tr>
<td>Recreation</td>
<td>0.7 (1.1)</td>
<td>5.3 (1.4)</td>
<td>1.0 (1.3)</td>
<td>0.7 (1.3)</td>
</tr>
<tr>
<td>Health</td>
<td>0.6 (1.0)</td>
<td>5.9 (1.6)</td>
<td>0.6 (1.0)</td>
<td>1.1 (1.8)</td>
</tr>
</tbody>
</table>
DALDA Questionnaire: Part B (Signs and Symptoms of Stress)

A main effect of response \((P < 0.001)\) and an interaction between training week and response \((P < 0.05)\) existed in Part B (signs and symptoms of stress) of the DALDA questionnaire. Post-hoc paired comparisons demonstrated that the weekly number of “B” (normal) responses were greater than the weekly number of “A” (worse than normal) and “C” (better than normal) responses across all weeks \((P < 0.05; d = 1.6 – 1.9)\) (Table 4-8). In addition, the weekly number of “A” (worse than normal) responses were greater than the number of “C” (better than normal) responses during Weeks 2, 3 and 4 \((P < 0.05; d = 1.2 – 1.4)\). Finally, participants reported a greater number of “A” and “C” responses during Weeks 2, 3 and 4 when compared to the Baseline week \((P < 0.05; d = 1.2 – 1.4)\) (refer to Figures 4-11, 4-12 and 4-13). During Week 4 (recovery), the number of “A” (worse than normal) responses were correlated with the decrease in the total weekly training load (kg) \((P < 0.05; R^2 = 0.63)\).

The most common changes in the signs and symptoms of stress were related to “Muscle Pains”, “Tiredness”, “Need for a Rest”, “Irritability”, “Weight”, “Unexplained Aches”, and “Enough Sleep” which all demonstrated increased “A” (worse than normal) response rates during Week 2 (overload), 3 (overload), and 4 (recovery), relative to the Baseline week \((P < 0.05)\) (Table 4-9). In addition, the participant’s reported “Techniques”, “Recovery Time”, “Between Sessions Recovery Time”, “Temper”, and “Swellings” were also negatively affected as the number of “C” (better than normal) responses declined during Week 3 (overload) and 4 (recovery), relative to the Baseline week \((P < 0.05)\) (Table 4-9). Finally, during Week 3 (overload) and 4 (recovery) participants reported fewer “C” responses for “Interest” when compared to Week 2 (overload) indicating a change in motivation towards weightlifting \((P < 0.05)\) (Table 4-9).
Table 4-8: Total weekly “A” (worse than normal), “B” (normal) and “C” (better than normal) responses for Part B (Signs and Symptoms of Stress) of the Daily Analysis of Life Demands of Athletes questionnaire. Data are presented as mean (± SD). *Significant difference from “B” (normal) response within week (P < 0.05); †Significant difference from “C” (better than normal) response within week (P < 0.05); ‡Significant difference in response from Baseline week (P < 0.05).

<table>
<thead>
<tr>
<th>Weekly Response</th>
<th>Baseline</th>
<th>Week 2 - Overload</th>
<th>Week 3 - Overload</th>
<th>Week 4 - Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.3 (11.1)*</td>
<td>48.3 (18.5)*‡</td>
<td>48.9 (19.9)*‡</td>
<td>42.4 (20.6)*‡</td>
</tr>
<tr>
<td>B</td>
<td>119.0 (13.8)</td>
<td>110.6 (22.0)</td>
<td>111.7 (24.4)</td>
<td>116.4 (26.1)</td>
</tr>
<tr>
<td>C</td>
<td>38.3 (12.0)*</td>
<td>16.1 (12.7)*‡</td>
<td>13.3 (17.9)*‡</td>
<td>16.0 (13.0)*‡</td>
</tr>
</tbody>
</table>

Figure 4-11: Changes in the Daily Analysis of Life Demands of Athletes Part B (signs and symptoms of stress) - “A” (worse than normal) scores during baseline, two weeks of overload training (grey) and one week of recovery training.
Figure 4-12: Changes in the Daily Analysis of Life Demands of Athletes Part B (signs and symptoms of stress) -“B” (normal) scores during baseline, two weeks of overload training (grey) and one week of recovery training.

Figure 4-13: Changes in the Daily Analysis of Life Demands of Athletes Part B (signs and symptoms of stress) -“C” (better than normal) scores during baseline, two weeks of overload training (grey) and one week of recovery training.
Table 4-9: Total weekly “A” (worse than normal), “B” (normal) and “C” (better than normal) responses for each “Signs and Symptoms of Stress” from Part B of the Daily Analysis of Life Demands of Athletes questionnaire. Data are presented as mean (± SD). *Significant difference from Baseline (P < 0.05); §Significant difference from Week 2 – Overload (P < 0.05); ¥Significant difference from Week 4 – Recovery (P < 0.05).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Baseline</th>
<th>Week 2 - Overload</th>
<th>Week 3 - Overload</th>
<th>Week 4 - Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Muscle Pains</td>
<td>0.4 (0.8)</td>
<td>3.1 (1.3)</td>
<td>3.4 (1.4)</td>
<td>4.0 (2.4)*</td>
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<tr>
<td>Techniques</td>
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<td>4.4 (1.3)</td>
<td>2.1 (1.3)</td>
<td>2.3 (1.7)</td>
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<tr>
<td>Tiredness</td>
<td>0.9 (1.2)</td>
<td>3.3 (1.4)</td>
<td>2.9 (0.7)</td>
<td>4.6 (1.9)*</td>
</tr>
<tr>
<td>Need for Rest</td>
<td>0.7 (0.8)</td>
<td>3.4 (1.0)</td>
<td>2.9 (0.9)</td>
<td>4.3 (2.1)*</td>
</tr>
<tr>
<td>Supplementary Work</td>
<td>1.1 (1.1)</td>
<td>5.6 (1.5)</td>
<td>0.3 (0.8)</td>
<td>0.6 (0.8)</td>
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<tr>
<td>Boredom</td>
<td>0.7 (1.3)</td>
<td>5.6 (1.6)</td>
<td>0.7 (1.0)</td>
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<td>Recovery Time</td>
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<td>3.3 (1.0)</td>
<td>3.1 (0.7)</td>
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<td>Irritability</td>
<td>1.3 (1.3)</td>
<td>4.7 (1.3)</td>
<td>1.0 (1.0)</td>
<td>3.0 (1.3)*</td>
</tr>
<tr>
<td>Weight</td>
<td>1.1 (1.2)</td>
<td>5.1 (1.8)</td>
<td>0.7 (1.9)</td>
<td>3.6 (3.4)</td>
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<tr>
<td>Throat</td>
<td>0.6 (1.0)</td>
<td>6.4 (1.0)</td>
<td>0.0 (0.0)</td>
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Table 4-9: Continued.

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<th>Factor</th>
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<th>Week 2 - Overload</th>
<th>Week 3 - Overload</th>
<th>Week 4 - Recovery</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Internal</td>
<td>0.7 (1.0)</td>
<td>5.1 (1.5)</td>
<td>1.1 (1.2)</td>
<td>1.4 (1.3)</td>
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<tr>
<td>Unexplained Aches</td>
<td>0.7 (1.0)</td>
<td>3.9 (0.9)</td>
<td>2.4 (1.0)</td>
<td>3.3 (1.4)*</td>
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<td>Technique Strength</td>
<td>0.9 (0.9)</td>
<td>3.9 (0.9)</td>
<td>2.3 (1.5)</td>
<td>2.0 (1.8)</td>
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<td>Enough Sleep</td>
<td>0.7 (0.5)</td>
<td>3.7 (0.8)</td>
<td>2.6 (0.8)</td>
<td>3.9 (1.6)*</td>
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<td>Session Recovery</td>
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<td>3.9 (1.1)</td>
<td>2.4 (0.5)</td>
<td>1.7 (2.4)</td>
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<td>General Weakness</td>
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<td>0.9 (1.5)</td>
<td>1.0 (2.2)</td>
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<td>Interests</td>
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<td>0.9 (1.5)</td>
<td>0.4 (1.1)</td>
</tr>
<tr>
<td>Arguments</td>
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<td>5.1 (1.8)</td>
<td>0.4 (0.5)</td>
<td>1.7 (1.5)</td>
</tr>
<tr>
<td>Skin Rashes</td>
<td>0.4 (0.8)</td>
<td>8.1 (3.9)</td>
<td>0.0 (0.0)</td>
<td>0.9 (2.3)</td>
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<td>0.6 (1.1)</td>
<td>1.9 (1.3)</td>
</tr>
<tr>
<td>Training Effort</td>
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<td>4.9 (1.3)</td>
<td>1.7 (1.3)</td>
<td>1.0 (1.4)</td>
</tr>
<tr>
<td>Temper</td>
<td>1.3 (0.8)</td>
<td>3.4 (1.1)</td>
<td>2.3 (1.0)</td>
<td>1.7 (1.6)</td>
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</table>
Table 4-9: Continued.

<table>
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<th>Factor</th>
<th>Baseline</th>
<th>Week 2 - Overload</th>
<th>Week 3 - Overload</th>
<th>Week 4 - Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Swelling</td>
<td>0.6 (1.0)</td>
<td>3.9 (1.8)</td>
<td>2.6 (1.9)</td>
<td>1.3 (2.2)</td>
</tr>
<tr>
<td>Likability</td>
<td>0.9 (0.7)</td>
<td>5.1 (1.5)</td>
<td>1.0 (1.2)</td>
<td>0.3 (0.5)</td>
</tr>
<tr>
<td>Running Nose</td>
<td>0.9 (1.1)</td>
<td>6.1 (1.1)</td>
<td>0.0 (0.0)</td>
<td>0.4 (0.8)</td>
</tr>
</tbody>
</table>
A main effect of week ($P < 0.05$) and mood state ($P < 0.001$) and an interaction between week and mood state ($P < 0.01$) existed for male and female weightlifters. Post-hoc paired comparisons demonstrated that Vigor and Fatigue scores were elevated when compared to Tension/Anxiety, Depression, Anger and Confusion scores during Week 2 (overload) and Week 4 (recovery) ($P < 0.05; d = 0.6 - 1.1$) (Table 4-10). Conversely, only Fatigue remained elevated when compared to all other mood states during Week 3 (overload) ($P < 0.05; d = 0.6 - 1.2$) (Table 4-10).

Total Mood Disturbance Scores (TMDS) increased by $18.5 \pm 19.8\%$ ($P < 0.05; d = 1.1$) from Week 2 (overload) to Week 3 (overload) and decreased by $13.4 \pm 9.5\%$ ($P < 0.05; d = 1.2$) from Week 3 (overload) to Week 4 (recovery) (Figure 4-11). Examination of the individual mood states revealed that the increase in the TMDS during Week 3 (overload) was due to elevated Depression ($P < 0.05; d = 0.8$), Anger ($P < 0.05; d = 0.5$) and Fatigue ($P < 0.05; d = 0.6$) scores (Table 4-10). Conversely, the decrease in the TMDS during Week 4 (recovery) was due to a decline in Depression ($P < 0.05; d = 0.8$) and Anger ($P < 0.05; d = 0.7$) scores (Table 4-10). The relative (percentage) changes in POMS scores were not correlated with the relative (percentage) changes in training load and/or performance measures between weeks.

**Table 4-10:** Mood state scores across the 2 week overload and 1 week recovery training periods. Data are presented as mean ($\pm$ SD). *Significant difference from vigor within week ($P < 0.05$); ‡Significant difference from fatigue within week ($P < 0.05$); §Significant difference in mood state from Week 2 - Overload ($P < 0.05$); ¶Significant difference in mood state from Week 3 - Overload ($P < 0.05$).
Figure 4-14: Total Mood Disturbance Scores across the 2 week overload and 1 week recovery training periods. Data are presented as mean (± SD). * Significant difference from Week 2 - Overload (P < 0.05); ‡ Significant difference from Week 4 - Recovery (P < 0.05).
4.5 Discussion

The purpose of this investigation was to identify and examine systemic responses that are associated with the recovery from and adaptation to prescribed, short-term variations in training load in competitive weightlifters. The major findings of this study were: (1) the microarray analyses identified 202 differentially expressed genes (3 h post-exercise) in the PBMCs of highly-trained competitive weightlifters. The transcriptional response 3 h post-exercise presented a strong genetic signature of immunological and inflammatory stress; (2) the selected candidate genes, CXCR4, CCL4 and DDIT4, exhibited a robust exercise-induced response (determined via qPCR) following the 2 wk overload, but not after the subsequent week of recovery training; (3) the 2 wk overload resulted in greater pre- and post-exercise plasma PC levels, indicative of enhanced tissue protein oxidation, which coincided with decrements in performance. Conversely, after the 1 wk of recovery training, pre- and post-exercise PC levels were significantly reduced in conjunction with a restoration of performance; (4) during the 2 wk overload participants reported a significant increase in the signs and symptoms of stress, and an increase in Total Mood Disturbance Scores (TMDS), indicating a negative affective response to the overload. Following the recovery week there was an improvement in these reported mood and stress parameters; and, (5) changes in maximal vertical jump and weightlifting performance were not associated with changes in plasma MIP-1β and PC levels and/or psychological indices.

To the best of our knowledge, this is the first investigation to document the PBMC transcriptional responses to HIRE in highly-trained competitive weightlifters. Furthermore, the results of this investigation extend previous research (Carlson et al., 2011; Mahoney et al., 2008) by examining the influence of variations in training load on the mRNA responses of selected candidate genes during a real-life training period in competitive athletes. The results of this investigation further our understanding of how competitive weightlifters respond and adapt to their intensive training structures that involve multiple daily HIRE sessions, along with repeated overload and recovery weeks.

In this investigation, the exercise intensities, total training volume per session (sets × repetitions) and the number of training sessions performed each week by the weightlifters exceeded the ACSM recommendations for improving strength and power (Ratamess et al., 2009). However, the increases in the weekly total training load (kg) and the number of
repetitions performed during Week 2 and 3 (overload) were comparable to those reported in previous investigations of hormonal and performance responses to short-term overload in competitive weightlifters (Fry et al., 1993; Pistilli et al., 2008; Warren et al., 1992). In addition, the percentage decreases in the training variables during the 1 wk recovery period were similar to those previously documented in periodized training programs for these athletes (Drechsler, 1998; Stone et al., 1999a, 1999b; 2006a).

From the 202 differentially expressed genes identified by the microarray analyses, CXCR4, CCL4 and DDIT4 were selected as the candidate genes of interest due to their involvement with known cell-to-cell interaction, immune cell trafficking and cell cycle signalling pathways (refer to Table 4-4). The preliminary microarray analyses were a critical step in this investigation as no literature existed on the PBMC transcriptional responses to HIRE in highly-trained strength and power athletes. It was anticipated that the greatest chance of detecting significant transcriptional changes would occur after the overload period as experienced resistance-trained adults require a far greater overload stimulus to induce significant increases in mRNA abundance, when compared with nonhabitual resistance trainers (Coffey et al., 2006a; 2006b). Thus, microarray analyses were performed on pre- and 3h post-exercise samples obtained at the end of the 2 wk overload period.

The robust immunological and inflammatory stress response demonstrated by the microarray data was also recently documented following a 30 min, moderate-intensity, moderate-volume resistance exercise protocol in recreationally-trained adults (Carlson et al., 2011). Additional similarities reported by Carlson et al. (2011) included an increased expression of genes associated with the “Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells” canonical pathway along with a significant increase in ORM1 mRNA expression (a plasma protein that is secreted during stressful conditions, injury and inflammation). These combined results demonstrate that resistance exercise disrupts cellular homeostasis resulting in a systemic inflammatory stress response which is evident after an acute bout of exercise in moderately-trained adults and after overload training in highly-trained competitive weightlifters.

Of particular interest in the current investigation were the exercise-induced responses of CXCR4 (3.4-fold change), CCL4 (1.6-fold change) and DDIT4 (1.4-fold change) following the overload period (Figure 4-5). The increased transcriptional responses of these genes coincided with decrements in maximal snatch (-4.3 ± 3.7%) and vertical jump (-7.2 ± 6.5%)
performance. In addition, the 2 wk overload induced a significant degree of muscle damage as indicated by the ~35% increase in basal plasma PC levels (Bloomer et al., 2005a; Dalle-Donne et al., 2003a; Lee et al., 2002) and the significant increase in “muscle pains”, “unexplained aches” and “swellings” as determined by Part B of the DALDA questionnaire (refer to Table 4-9). Conversely, no exercise-induced responses were detected for the candidate genes following the recovery period, during which time a lower indication of tissue damage was evident and performance was restored.

The damage sustained to skeletal muscle, connective tissue, and/or bones and joints following high-intensity exercise has previously been referred to as “adaptive micro-trauma” (Smith, 2000a; 2000b). The adaptive micro-trauma results in an acute inflammatory response, with the final purposes of repair, regeneration and subsequent adaptation of the injured tissue (Clarkson et al., 1988; 2002; MacIntyre et al., 1995). In accordance with these findings, the exercise-induced muscle damage that was sustained following the 2 wk overload period resulted in a systemic inflammatory response that was represented by a 3.4-fold increase in CXCR4 mRNA expression in PBMCs.

CXCR4 is functionally expressed on a number of cell types, some of which reside in bone marrow and are mobilized into the circulation in response to injury or infection (e.g. neutrophils and tissue-specific stem cells) (Kucia et al., 2004; Martin et al., 2003; Ratajczak et al., 2003a). In the current investigation, changes in CXCR4 mRNA were quantified in isolated lymphocyte and monocyte populations. Despite CXCR4’s diverse expression it selectively binds to one ligand, Stromal Cell-Derived Factor-1 (SDF-1) (Busillo et al., 2007). In response to injury, SDF-1 expression has been shown to increase within regions of damaged tissue (Kucia et al., 2004b; Ratajczak et al., 2006). The resulting SDF-1 chemoattraction gradient draws circulating CXCR4⁺ cells to the site of damage thereby enhancing the potential for tissue repair and regeneration (Kucia et al., 2004b; Ratajczak et al., 2006). In addition, muscle damaging exercise protocols (that led to decreased muscular performances) have resulted in increased leukocyte infiltration within skeletal muscle (Fielding et al., 1993; MacIntyre et al., 1996). Thus, it appears that the increased CXCR4 mRNA expression in lymphocytes and monocytes following overload is priming these cells to follow an SDF-1 chemoattraction gradient that is geared towards subsequent tissue infiltration and repair (Arnold et al., 2007; Sánchez-Martín et al., 2011). As this is the first investigation to document an increased expression of CXCR4 mRNA in PBMCs following
HIRE in highly-trained athletes, further investigations need to be undertaken to include the expression of SDF-1 in muscle following similar exercise in resistance-trained adults.

The exercise-induced increase in CCL4 mRNA expression following overload adds further evidence to the likelihood that muscle damage occurred during this period as enhanced CCL4 expression is a characteristic feature of injured skeletal muscle (Hirata et al., 2003; Summan et al., 2003b; Warren et al., 2004). Furthermore, CCL4 plays an important role in muscle repair, regeneration and subsequent growth (Warren et al., 2004; Yahiaoui et al., 2008) as it is involved with the chemoattraction of inflammatory cell types (Menten et al., 2002; Rollins, 1997) and the regulation of myoblast proliferation (Yahiaoui et al., 2008). The 1 wk recovery period resulted in no discernible exercise-induced response of CCL4 in conjunction with a decrease in tissue damage indices (refer to Figure 4-7) and a restoration of performance. Collectively, these results suggest that an increase in CCL4 mRNA expression in the PBMCs of competitive weightlifters occurs as a function of the exercise-induced muscle damage that is sustained during short-term periods of overload.

Plasma MIP-1β levels were shown to decrease in a similar fashion from pre- to post-exercise following the overload and recovery training periods (refer to Figure 4-6). These findings are likely explained by a resistance-exercise induced movement of fluid and plasma proteins from the circulation into other tissues (Smith, 2000a). Previous investigations into the plasma response of MIP-1β to exercise have produced some equivocal results. Evaluations of the effects of aerobic exercise have demonstrated significant increases (1.2 - 4.1-fold) (Nieman et al., 2005b; Ostrowski et al., 2001), or no change (Ostrowski et al., 1998) in plasma MIP-1β levels. Furthermore, no changes in plasma MIP-1β have been reported following acute bouts of HIRE (Paulsen et al., 2005; Risøy et al., 2003). As the gene and protein expression responses of CCL4/MIP-1β were investigated during the early recovery period (i.e. 3 h post-exercise), it is possible that circulating levels of MIP-1β may have peaked at a later stage of recovery in competitive weightlifters. Thus, further investigation is required at multiple post-exercise time points to clarify if MIP-1β plays a significant role in subsequent muscle repair and/or regeneration processes following HIRE in well-trained adults.

In the current investigation, DDIT4 demonstrated a significant exercise-induced transcriptional response following the 2 wk overload. Such findings are relevant to strength and power athletes as DDIT4 is a major stress-responsive negative regulator of the
mammalian target of rapamycin (mTOR) signalling pathway (Drummond et al., 2009a; Ma et al., 2009). Skeletal muscle repair, regeneration and subsequent growth are associated with the activation of mTOR signalling as this pathway is involved with the modulation of protein synthesis post-exercise (Deldicque et al., 2005; Drummond et al., 2009b; Ma et al., 2009; Miyazaki et al., 2009). Previous evidence has shown that DDIT4 expression is upregulated in response to cellular stressors including hypoxia, muscle contraction and the administration of glucocorticoids (Drummond et al., 2008b; Wang et al., 2006). In addition, DDIT4 is upregulated during periods of energy stress and starvation (Drummond et al., 2008a; Sofer et al., 2005). Although resistance has been shown to have ~66% of the energy requirement of vigorous aerobic exercise of the same duration (Alexander, 2002), the indirect effects of HIRE are evident through increases in excess post-exercise oxygen consumption (EPOC) and resting metabolic rate (Alexander, 2002; Melby et al., 1993). High-intensity, intermittent resistance exercise produces a greater EPOC response when compared to low-intensity resistance exercise of an equal work volume (Thornton et al., 2002) and/or continuous aerobic exercise of a similar exercise oxygen uptake (Burleson Jr et al., 1998). Thus, appropriate post-exercise feeding interventions are of great importance in attenuating the increased energy demands of HIRE overload training. Of particular interest was that during the overload period, participants reported that their “diet” and “weight” were deemed to be “worse than normal” (according to their DALDA responses) suggesting an increased energy stress. Furthermore, as a strong association exists between Total Mood Disturbance Scores (TMDS) and circulating cortisol levels (Cruess et al., 2000; McGhee et al., 2009; McKinney et al., 1997; O’Connor et al., 1989), it is likely that the 18.5 ± 19.8% increase in TMDS during the overload coincided with an increase in glucocorticoid activity resulting in the enhanced expression of DDIT4 mRNA (McGhee et al., 2009; Wang et al., 2006). No such exercise-induced response for DDIT4 was evident following the 1 wk recovery period during which time “diet”, “weight” and TMDS had improved (refer to Table 4-7 and 4-9 and Figure 4-14). Therefore, although the results of the current investigation demonstrate that the potential to recruit inflammatory cells involved in muscle repair and regeneration is enhanced during overload (i.e. via the increased CXCR4 and CCL4 mRNA expression), a DDIT4-mediated inhibition of mTOR signalling may hinder subsequent anabolic processes in competitive weightlifters. As the regulation of protein synthesis is essential to maintain the phenotype for improved muscular force production (Harmon et al., 2010; Hawley et al., 2010), it is evident that the training stress and dietary intakes of competitive weightlifters
must be closely monitored during periods of overload to enable positive adaptations to take place.

As no exercise-induced responses occurred for CXCR4, CCL4 and DDIT4 following the 1 wk of recovery, it appears that highly-trained competitive weightlifters require short-term periods of overload to elicit the gene expression responses that lead to adaptation. These findings are supported by previous evidence demonstrating that experienced resistance-trained adults require a far greater overload stimulus to induce significant increases in mRNA abundance, when compared with nonhabitual resistance trainers (Coffey et al., 2006a; 2006b). Therefore, these combined results provide a physiological justification for the prescription of structured overload and recovery periods in competitive weightlifters.

Previous research has demonstrated that the POMS correlates well with overreaching and overtraining syndromes where athletic performance, mood and well-being are compromised (acutely or chronically) in response to brief (days) or sustained (weeks) periods of intensified training (Fry et al., 1994d; Halson et al., 2002; 2004; Morgan et al., 1988). On this basis, we hypothesized that during the 2 wk overload, decrements in performance would be associated with an increase in TMDS. However, no such correlations existed in the current investigation. The lack of correlations between PC levels, psychological indices and performance measures are likely due to: 1) the relatively minor performance decrements, as reflected by their moderate to small effect sizes ($d = 0.1 - 0.3$); and 2) a decreased stress response to the short-term overload period due to prior exposure to this type of training structure (Fry et al., 1994a; Kraemer et al., 1998c). It was anticipated that acute changes in maximal snatch and vertical jump performance would be interrelated as a strong kinematic relationship has been shown to exist during the propulsive phases of these two movements (Canavan et al., 1996; Carlock et al., 2004; LeFavi et al., 2011). In support of these previous findings, estimated vertical jump peak power (W) was strongly correlated with snatch performance across all time points ($P < 0.001; R^2 = 0.9$).

The DALDA questionnaire was designed and validated to identify the sources and symptoms of stress in elite-level athletes (Rushall, 1990). At the onset of the 2 wk overload period, participants reported a greater number of “A” (worse than normal) responses for both the signs (Part A) and symptoms (Part B) of stress (refer to Figures 4-8 and 4-11). As mood oscillates with training and recovery, the point at which negative mood disturbance scores
remain elevated for 4 or more consecutive days can be used to identify a state of overreaching (as described in Section 2.6.4) (Halson et al., 2002; Rushall, 1990). As the increased frequency of self-reported “A” (worse than normal) responses persisted throughout the entire 2 wk overload period, the competitive weightlifters were deemed to be “overreached” (refer to Figures 4-8 and 4-11). Furthermore, this statement is supported by the coincident short-term decrements in performance that occurred during the overload period which is another hallmark characteristic of overreached athletes (refer to Section 2.6.4).

Of particular interest and relevance to this investigation is the previously reported link between systemic inflammation, arising from sustained periods of high-volume, high-intensity exercise (i.e. overload), and the affective responses to training. According to the psychoneuroimmunological model (Maes, 1995; Maier et al., 1998; Smith, 1991), inflammatory molecules can act upon the central nervous system resulting in an array of symptoms including a reduced appetite, weight loss, reduced thirst, reduced libido, depression, loss of interest, fear, and sleep disturbances (Smith, 2000a). In the current investigation, the most common changes in the sources of stress during overload were related to “Sleep” and “Diet” which demonstrated increased “worse than normal” response rates. Furthermore, the most common negative changes in the signs and symptoms of stress during overload were related to “Muscle Pains”, “Tiredness”, “Need for a Rest”, “Irritability”, “Weight”, “Unexplained Aches”, and “Enough Sleep”. Finally, as increases in TMDS were attributed to increases in “Depression”, the collective results of the POMS and DALDA questionnaires support the psychoneuroimmunological model of stress.

Collectively, the results have demonstrated that 2 wk of overload resulted in significant immunological and inflammatory stress and an increase in skeletal muscle damage contributing to performance decrements and negative behavioral changes in competitive weightlifters (refer to Figure 4-15). However, a subsequent week long recovery period of reduced training was sufficient for these negative physiological, performance and affective responses to resolve. According to Stone and Fry (1998), periods of overload should not last longer than 3 wk in strength and power athletes, with a restoration, followed by a delayed increase in performance occurring approximately 2-5 wk upon a reduction in training stress. As performance variables were restored to baseline levels in competitive weightlifters following 1 wk of recovery, it is likely that performance gains would have occurred following an additional week of reduced training (refer to Figure 4-15). Such a training
approach is followed when tapering for a competition. However, upon completion of the 4 wk study period (baseline to recovery), the participants began another 2 wk overload and 1 wk recovery training phase as part of their planned competition training cycle. Therefore, further research is warranted in the area of pre-competition tapering to determine what physiological variables contribute to enhanced competitive performance in highly-trained weightlifters.
Figure 4-15: Summary of events that occur following 2 weeks of overload and 1 week of recovery training in competitive weightlifters. It is proposed that performance improvements will occur following an additional reduction in training (tapering) but this has yet to be examined.
Technical Considerations

Resistance exercise is known to produce transient changes in total leukocyte count. Such changes are dependent upon the exercise volume and intensity, the duration of the rest intervals and exercise session, and the nutritional status of the participant (Mayhew et al., 2005; Nieman et al., 1992; Pedersen et al., 1998; Risøy et al., 2003). Although immunological parameters generally return to baseline levels within 2-3 h following resistance exercise (Mayhew et al., 2005; Natale et al., 2003; Nieman et al., 2004; Simonson et al., 2004b; Tvede et al., 1989), it is possible that the mRNA results obtained 3 h post-exercise were influenced by variations in lymphocyte and monocyte count. However, since the training sessions were replicated on the performance testing days (Fridays) and the subsequent biological sample collection days (Saturdays), along with the pre- and post-exercise feeding conditions, any potential variations in the cellular composition of peripheral blood would have been negligible (during sample acquisition) across the overload and recovery periods. A second limitation in this investigation is the small sample size used for the microarray analyses. However, the central purpose of the microarray data was to identify candidate genes for further analyses via qPCR as no literature currently exists on the PBMC transcriptional responses to HIRE in competitive strength and power athletes. Future investigations are warranted using this technology in a larger cohort of well-trained adults following HIRE. Finally, although the assessment of the competitive lifts by trained weightlifters is highly reliable (McGuigan et al., 2004), this performance measure does not provide physiological data on changes in neuromuscular function (i.e. peak force, contractile rate of force development and/or contractile impulse). As the measurement of contractile parameters may reflect injury-induced functional impairments (Warren et al., 1999), the development of a reliable, valid, unbiased and non-technique dependent test to quantify neuromuscular function in weightlifters is required.
4.6 Conclusions

To the best of our knowledge, this is the first investigation to identify and examine the PBMC transcriptional responses to HIRE in highly-trained weightlifters. As hypothesized, cDNA microarray analyses detected the differential expression of a number of genes in the PBMCs of competitive weightlifters following overload training. Follow up qPCR analyses demonstrated that a robust exercise-induced response of the selected candidate genes, CXCR4, CCL4 and DDIT4, occurred after overload but not recovery training. Furthermore, performance decrements observed during the overload period coincided with increases in indices of tissue damage and self-reported adverse signs and symptoms associated with stress in the athletes. The subsequent recovery period resulted in a restoration of performance, a decrease in tissue damage and an improvement in reported mood and stress parameters. Therefore, these findings suggest that cycles of overload and recovery training are essential to induce gene expression changes that lead to functional adaptations in these highly-trained athletes.

As the HIRE performed by competitive weightlifters induced a robust increase in CXCR4 mRNA expression in circulating PBMCs, further research needs to be undertaken to include the expression of SDF-1 in skeletal muscle following similar exercise protocols. In addition, other inflammatory cytokines/chemokines with known links to muscle repair and regeneration pathways (e.g. MCP-1) need to be investigated following HIRE. Such research will enhance our understanding of both the short-term responses and long-term adaptations that lead to the restoration and improvement of neuromuscular performance. Finally, as increased energy stress and nutrient availability is known to influence the gene expression responses to exercise, the influence of post-exercise feeding following high-power, HIRE needs to be pursued in trained adults.
5.0 DIVERGENT MUSCLE FUNCTIONAL AND ARCHITECTURAL RESPONSES TO TWO SUCCESSIVE HIGH INTENSITY RESISTANCE EXERCISE SESSIONS IN COMPETITIVE WEIGHTLIFTERS AND RESISTANCE TRAINED ADULTS

5.1 Abstract

Peak force (PF), contractile rate of force development (RFD) and contractile impulse (CI) are of great importance to competitive weightlifters. These athletes routinely perform successive bouts of high-intensity resistance exercise (HIRE) within the same day (double-day training) with the aim of improving muscular function and weightlifting performance. The purpose of this investigation was to determine and compare the PF, contractile RFD and CI responses to double-day training between weightlifters and resistance-trained adults (n = 16 per group). Furthermore, we sought to establish whether acute changes in muscle function were associated with acute changes in muscle architecture. Isometric front squat PF, contractile RFD, CI and the pennation angle (θp), anatomical and physiological thickness of the m. vastus lateralis (VL) were determined before and after two equivalent HIRE sessions separated by 4-6 h rest. Each session consisted of 10 single repetitions of the dynamic barbell front squat interspersed with 2 min rest, using a load equivalent to 90% of the pre-Session PF. Weightlifters demonstrated greater PF at all time points when compared to resistance-trained adults and exhibited no significant within or between session changes in PF, contractile RFD or CI. Conversely, resistance-trained adults demonstrated within- and between-session decreases in PF and between-session increases in contractile RFD and CI. As no correlations were found between the relative within-session changes in muscle function and the concomitant changes in muscle architecture, other factors must contribute to the divergent responses in PF, contractile RFD and CI between weightlifters and resistance-trained adults.
5.2 Introduction

Competitive weightlifting is a strength and power sport in which two, multi-joint, whole body movement lifts are performed in competition; the snatch and the clean and jerk. During the performance of these lifts, weightlifters have demonstrated some of the highest absolute and relative maximal power outputs reported in the literature (Garhammer, 1980, 1991). Maximal voluntary isometric peak force (PF) is strongly related to weightlifting performance and other dynamic muscle actions (Haff et al., 2005; Hakkinen et al., 1986b; Stone et al., 2003a; 2005). However, while PF is reached in the vicinity of 300 – 600 ms (Aagaard et al., 2002; Thorstensson et al., 1976b; Zatsiorsky, 2003), weightlifters achieve maximum barbell velocities and peak power outputs in <260 ms (Campos et al., 2006; Garhammer, 1991; Gourgoulis et al., 2000; 2009). Thus, the contractile rate of force development (RFD), defined as the slope of the force-time curve (Aagaard et al., 2002), is of great importance to weightlifters (Haff et al., 2005). Furthermore, the contractile impulse (CI), defined as the integral of the force-time curve (Aagaard et al., 2002), is another measure of muscular function relevant to weightlifting performance. Contractile impulse factors in the overall influence of the various time-related contractile RFD parameters (Aagaard et al., 2002; Baker et al., 1994). As mass remains constant during the performance of a weightlifting movement, an increase in CI would be associated with a higher movement velocity, a decrease in movement time and a greater displacement of the loaded barbell (Garhammer et al., 1992; Schilling et al., 2008). To enhance muscular function and competitive performance, the training practices of weightlifters often include successive high-intensity resistance exercise (HIRE) sessions that are performed within the same day. The muscle functional responses of weightlifters to each session may explain how these athletes are able to sustain high loads during successive training sessions.

To improve muscular strength and power, the American College of Sports Medicine recommends performing 4-6 sessions.wk\(^{-1}\) of moderate [30-60% of 1 repetition max (1RM)] to high intensity (≥80% 1RM) resistance exercise. However, it is common for strength and power athletes to divide a given training volume across two sessions that are performed on the same day. Such “double-day” protocols produce significantly greater increases in muscular strength, hypertrophy and maximal neural activation of the trained musculature when compared to performing the same training volume across one daily session (Häkkinen et al., 1994a; Hartman et al., 2007). It is known that PF declines in resistance-trained adults
when resistance exercise is performed twice during the same day (Chiu et al., 2004). Conversely, the RFD does not appear to be compromised in well-trained adults following two successive resistance exercise sessions of differing intensity and modality (Chiu et al., 2004; Häkkinen et al., 1988a; 1992). To the best of our knowledge, no investigation has examined the acute CI responses to double-day training. Consistent with these findings, double-day programs are often split according to muscle group (e.g. upper vs. lower body) and training intensity (e.g. high vs. low) to enable recovery between subsequent sessions (Häkkinen et al., 1994a; Kraemer et al., 1998a). However, weightlifters are known to perform two or more HIRE sessions per day, inclusive of exercises for the same major muscle groups, six or seven days per week (Garhammer et al., 2003; Stone et al., 2006a; Zatsiorsky, 1995). As certain kinematic parameters (e.g. maximum barbell acceleration, velocity and displacement) must be achieved in order to successfully perform weightlifting movements (Garhammer, 1985, 1991, 1993), we anticipate that PF, contractile RFD and CI are maintained (or enhanced) in weightlifters during successive HIRE bouts. It has been proposed that the maintenance (or enhancement) of muscular function within and between bouts of HIRE may be attributable to acute changes in the architecture of skeletal muscle (Mahlfeld et al., 2004; Tillin et al., 2009).

Peak force, contractile RFD and CI are influenced by a number of morphological factors including muscle fiber type, tendon properties, pennation angle ($\theta_p$) and muscle thickness (Blazevich et al., 2007; Cormie et al., 2011a; Kawakami et al., 1995). Pennation angle ($\theta_p$), defined as the angle of the muscle fascicles relative to the points of insertion at the tendon or aponeurosis determines the arrangement of sarcomeres within a muscle (Aagaard et al., 2001; Narici, 1999). A large anatomical $\theta_p$ allows more sarcomeres to be arranged in parallel. This in turn increases muscle thickness and PCSA (i.e. the cross-sectional area perpendicular to the line of fascicles) which are both positively associated with PF (Blazevich et al., 2007; Cormie et al., 2011a; Narici, 1999). In addition, muscle fibers of a greater $\theta_p$ operate closer to their optimum length which is advantageous to force production (Blazevich, 2006). Conversely, smaller anatomical $\theta_p$ allow more sarcomeres to be arranged in series which facilitates a rapid transmission of force to the tendon, thus increasing contractile RFD and CI (Fukunaga et al., 1997; Gans et al., 1991; Kawakami et al., 1993; Kumagai et al., 2000).

Acute changes in $\theta_p$ occur in response to the recent history of previous muscular contractions. These changes appear to be influenced by the total exercise volume. High-power dynamic resistance exercise performed to failure and a high volume of isometric maximal voluntary
contractions (MVC) have been shown to acutely increase $\theta_p$ by 10-11%, respectively (Csapo et al., 2011; Kubo et al., 2001). In contrast, an acute decrease in $\theta_p$ of ~11% has been reported 3-6 min following 3 isometric MVC (Mahlfeld et al., 2004). Theoretically, an increased post-contraction $\theta_p$, and consequently an increased PCSA, would increase PF. Conversely, a post-contraction reduction in $\theta_p$ may provide a mechanical advantage for rapid force transmission to the tendon, thereby improving contractile RFD and CI. However, no investigation to date has determined the functional significance of such changes in skeletal muscle architecture.

The first objective of this investigation was to determine and compare the PF, contractile RFD and CI before and after each of two equivalent HIRE sessions performed within the same day between weightlifters and resistance-trained adults. The second objective was to establish whether acute changes in PF, contractile RFD and CI were associated with acute changes in $\theta_p$ or thickness of the m. vastus lateralis (VL). It was hypothesized that: 1) PF, contractile RFD and CI would decrease within and between the two HIRE sessions in the resistance-trained adults only and 2) the maintenance and/or enhancement of PF in weightlifters would be associated with an acute relative increase in VL $\theta_p$ and subsequently muscle thickness.
5.3 Methods

5.3.1 Participants

Sixteen competitive weightlifters and sixteen resistance-trained adults volunteered to participate (Table 5-1). As a requirement of the study, all participants were free from acute or chronic injury at the time of data collection and were not using any performance enhancing supplements (e.g. creatine) or banned substances (as per the 2010 World Anti-Doping Code). Data was obtained from the weightlifters during a normal training phase (i.e. not during a competition peaking phase) during which they performed 8.0 ± 0.8 training sessions per week. Nine weightlifters were New Zealand National record holders at the time of data collection. Resistance trained adults were required to have performed regular resistance exercise (i.e. more than once per week for the previous year), and were able to perform a free-weight barbell front squat to a parallel depth (defined as the top of the thighs being parallel to the ground) with a weight equivalent to or greater than their body mass. All participants provided written informed consent prior to commencing. The investigation was approved by the University of Auckland Human Participants Ethics Committee.

5.3.2 Experimental Design

Each participant was instructed to arrive at the facility in the morning in a rested and fed state. After obtaining initial (pre-Session) ultrasound images of the VL, participants performed a standardized warm up, followed by three isometric MVC in the front squat position during which muscle function was measured. Participants were then required to complete a HIRE session consisting of a dynamic warm-up and 10 high intensity dynamic front squats with 2 min rest between repetitions. Post-Session ultrasound images were obtained directly after the tenth front squat and the muscle function testing was repeated. The complete sequence was repeated 4-6 h later.

5.3.3 Muscle Architecture

A 7.5 MHz linear array transducer (Chison 8300 Digital Ultrasound System, China) was used to obtain sagittal plane ultrasound images of the VL. This widely used imaging technique is sensitive to small changes in skeletal muscle architecture (Loram et al., 2006) and has proven
suitable for the non-invasive quantification of healthy and diseased pennate muscle (Pillen et al., 2007; Walker et al., 2004). Participants lay supine with their right leg supported by a custom-built A-frame structure which positioned the knee at an angle of 45°. This position has been shown to reduce fascicle curvature and improve the reliability of repeated measurements, whilst enabling the detection of inter-individual differences in muscle architecture (Blazevich et al., 2007). The probe was applied mid-thigh such that images were recorded at 50% of femur length. Water-soluble transmission gel was used between the probe and skin surface to aid in acoustic contact. Minimal consistent pressure of the probe was applied to avoid compression of the muscle. Muscle thickness and $\theta_p$ were determined from the images using digitizing software (Scion Image for Windows, Scion Corporation, MD, USA). Pennation angle was defined as the angle between the echos of the interfaces between the muscle fiber bundles and the deep aponeurosis (Aagaard et al., 2001). Anatomical muscle thickness was determined as the mean of duplicate measurements of the perpendicular distance between the superficial and deep aponeuroses (Figure 5-1). Physiological muscle thickness was calculated as the square root of (anatomical muscle thickness$^2 + [\tan \theta_p, \text{anatomical muscle thickness}]^2$) (Blazevich et al., 2007).
**Figure 5-1**: Sagittal plane ultrasound image of the vastus lateralis recorded at 50% femur length with the knee positioned at 45°. Anatomical muscle thickness was measured as the perpendicular distance between the superficial and deep aponeuroses. Muscle fiber pennation angle ($\theta_p$) was measured as the angle between the VL muscle fascicles and the deep aponeurosis.

### 5.3.4 Muscle Function

All force-time curve analyses were conducted using a calibrated 59.5 x 89.5 cm force plate (Kelba Onspot Power Plate, Sydney, Australia) placed within a custom-set up power rack with safety bars (Figure 5-2).
Participants performed a warm up of 10 dynamic front squats to a parallel depth using a standard 20 kg barbell, followed by 10 repetitions with 50% of their recently attained 1 repetition maximum. Participants were then required to assume a stationary front squat to parallel depth (with a standard barbell) on the force plate to ‘zero’ the system. A second set of safety bars were inserted into the power rack at a height that enabled participants to perform an isometric front squat at a parallel thigh position (Figure 5-2). Participants were instructed to raise the barbell so it ‘lightly’ touched the upper safety bars, and upon the count of three, performed an isometric contraction at ~50% of their maximum perceived effort for three seconds. This familiarization step was repeated at ~80% of the participant’s maximum perceived effort. Following the familiarization, the participants completed three isometric MVC. Participants were instructed to push as hard and as fast as possible and each attempt was separated by a 1 minute rest period.

All data was sampled at 1,000 Hz (Chart™ V5.5.5, AD Instruments) with a low-pass digital filter applied with a cut-off frequency set at 15 Hz. The procedure generated a force-time curve from which PF, contractile RFD, peak RFD and CI were calculated. Contractile RFD, peak RFD and CI were determined from the trial exhibiting the highest PF. Contractile RFD, peak RFD and CI were calculated. Contractile RFD, peak RFD and CI were determined from the trial exhibiting the highest PF.
Peak force, contractile RFD and CI are routinely determined using isometric contractions (Blazevich et al., 2008; Chiu et al., 2004; Häkkinen et al., 1988a; 1992). The effectiveness of isometric measurements to assess dynamically induced training adaptations can be enhanced by selecting a body position specific to the dynamic performance of interest (Haff et al., 1997; Stone et al., 2003a; Wilson et al., 1996). The isometric front squat was chosen due to its similarity with the clean phase of the competitive clean and jerk and other complementary exercises that are used by weightlifters and resistance-trained adults (i.e. dynamic front squat and power clean). Furthermore, as maximal muscle activation is enhanced during isometric bilateral versus unilateral contractions (Behm et al., 2003), we were confident that this testing procedure would provide a valid representation of the PF, contractile RFD and CI responses following the dynamic bilateral exercise sessions (Aagaard et al., 2002; Baker et al., 1994).

5.3.5 Exercise Sessions

Each exercise session was comprised of 10 dynamic parallel front squats (with 2 min rest between each repetition) with a load equivalent to 90% of the participant’s pre-Session PF. Before starting each exercise session, participants performed three warm-up front squats sets (3 repetitions at 65%, 2 repetitions at 75% and 1 repetition at 85% of the equivalent pre-
Session PF) with the depth of each repetition confirmed visually by an investigator. The warm-up protocol was of a low volume to minimize any fatigue prior to the exercise session. The two exercise sessions were separated by 4-6 hr rest in accordance with previous investigations (Chiu et al., 2004; Häkkinen et al., 1988a; 1992).

5.3.6 Data Presentation and Statistics

Data are presented as mean ± SE. Independent samples t-tests were used to assess differences between the descriptive characteristics of weightlifters and resistance-trained adults. Where data did not follow a normal distribution according to Shapiro-Wilk testing, analyses were performed on log-transformed data. Two factor group (weightlifters, resistance-trained) by time (pre-Session 1, post-Session1, pre-Session 2, post-Session 2) repeated-measures ANOVA was used to investigate each variable (PF, contractile RFD, relRFD, CI, \( \theta_p \) and muscle thickness). The analyses tested for main effects of group and time and any interaction between group and time. Where a significant main effect was found by ANOVA, post-hoc paired comparisons were made using the method of Student-Newman-Keuls. Effect sizes were calculated according to the method of Cohen where \( d = 0.8 \) is considered a large effect, \( d = 0.5 \) is moderate, and \( d = 0.2 \) a small effect size (Cohen, 1992). To test for an association between skeletal muscle function and architecture, Pearson product moment correlations were used to evaluate: 1) pre-Session PF and VL \( \theta_p \) and, 2) the relative within-Session changes in PF, contractile RFD, relRFD and CI and the relative within-Session changes in VL \( \theta_p \) and muscle thickness. Statistical significance was set at \( P < 0.05 \). Data were analyzed with SigmaPlot 11.0 statistical software (Chicago, IL).
5.4 Results

5.4.1 Participants

The mean body mass did not differ between the groups, whereas the weightlifters were younger \((P < 0.001; \ d = 1.1)\) and shorter than resistance-trained adults \((P < 0.05; \ d = .77)\) (Table 5-1). All participants were able to complete the exercise sessions as per the prescribed protocol, thereby enabling all repetitions within a session to be performed at the same relative load \((90\% \text{ of Pre-session PF})\).

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Mass (kg)</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ((N = 16))</td>
<td>21.5 ± 1.0</td>
<td>81.54 ± 6.3</td>
<td>1.68 ± 0.02</td>
</tr>
<tr>
<td>WL Men ((N = 13))</td>
<td>20.7 ± 1.0</td>
<td>85.54 ± 7.3</td>
<td>1.71 ± 0.02</td>
</tr>
<tr>
<td>WL Women ((N = 3))</td>
<td>25.0 ± 3.2</td>
<td>64.67 ± 5.7</td>
<td>1.56 ± 0.03</td>
</tr>
<tr>
<td>RT All ((N = 16))</td>
<td>26.2 ± 0.8**</td>
<td>75.51 ± 2.9</td>
<td>1.75 ± 0.02*</td>
</tr>
<tr>
<td>RT Men ((N = 13))</td>
<td>26.5 ± 0.9**</td>
<td>79.13 ± 2.6</td>
<td>1.78 ± 0.02*</td>
</tr>
<tr>
<td>RT Women ((N = 3))</td>
<td>24.7 ± 1.8</td>
<td>59.80 ± 3.2</td>
<td>1.65 ± 0.05</td>
</tr>
</tbody>
</table>

5.4.2 Peak Force

Main effects of group \((P < 0.001)\), time \((P < 0.001)\) and an interaction between group and time existed for PF \((P < 0.01)\) (Figure 5-3). Post-hoc paired comparisons showed that the PF in weightlifters was higher when compared to resistance-trained adults at all time points \((P < 0.001; \ d = 1.11 – 1.24)\) (Fig. 4). In addition, weightlifters demonstrated no significant within- (Session 1: \(P = 0.49; \ d = 0.07\); Session 2: \(P = 0.08; \ d = 0.25\)) or between-session (\(P = 0.22; \ d = 0.24\)) differences in PF. Conversely, PF in the resistance-trained adults decreased by \(16.0 ± 3.0\%\) within Session 1 \((P < 0.001; \ d = 0.64)\) and by \(12.2 ± 2.8\%\) within Session 2 \((P = 0.002; \ d = 0.51)\).
Furthermore, PF in resistance-trained adults pre-Session 2 was 7.0 ± 2.6% lower than in pre-Session 1 ($P < 0.05; d = 0.28$).

**Figure 5-3:** Mean (SE) pre- and post-Session peak force relative to body mass (N/kg) in weightlifters (♦) and resistance trained adults (■) for two high intensity resistance exercise sessions performed on the same day. *Significant difference between Sessions for group ($P < 0.05$); **Significant difference within Session for group ($P < 0.01$); ‡Significant difference between groups at the same time point ($P < 0.001$).

### 5.4.3 Contractile Rate of Force Development

Contractile RFD was greater in weightlifters than in resistance-trained adults at 0-100, 0-200 and 0-400 ms ($P < 0.05$). There were also main effects of time at 0-30 ms ($P < 0.05$) and an interaction between group and time at 0-200 ms ($P < 0.05$) (Figure 5-4). Post-hoc comparisons showed that pre-Session 1 contractile RFD was 22.6 – 52.3% higher in weightlifters when compared to resistance-trained adults at 0-30 ($P = < 0.05; d = 0.26$), 0-100 ($P = < 0.05; d = 0.88$), 0-200 ($P = < 0.001; d = 1.09$) and 0-400 ms ($P = < 0.05; d =
No significant within- or between-session differences in contractile RFD were observed for weightlifters. Conversely, contractile RFD from 0-400 ms in the resistance-trained adults decreased by 22 ± 6.8% within Session 2 (P < 0.05; d = 0.7). In addition, contractile RFD in the resistance-trained adults pre-Session 2 was higher than the respective pre-Session 1 values at 0-30 (P < 0.05; d = 0.34) and 0-200 ms (P < 0.05; d = 0.57). For relRFD, main effects of time were observed at 0-30 ms (P < 0.05) and group × time interactions were evident at 0-100, 0-200 and 0-400 ms (P < 0.05). Post-hoc paired comparisons showed that the relRFD pre-Session 1 was 33.3 ± 13.1%, 25.6 ± 15.7% and 15.8 ± 7.8% higher in weightlifters when compared to resistance-trained adults at 0-100 ms (P = < 0.05; d = 0.60), 0-200 ms (P = < 0.001; d = 0.86) and 0-400 ms (P = < 0.05; d = 0.85), respectively. No significant within- or between-session differences in relRFD were observed for weightlifters. Conversely, resistance-trained adults demonstrated significant within- (pre-post Session 1) (P = < 0.05; d = 0.20 – 0.85) and between-session (pre-Session 1 – pre-Session 2) (P = < 0.05; d = 0.49 – 0.87) increases in relRFD at 0-30, 0-100, 0-200 and 0-400 ms.
Figure 5-4: Mean (SE) pre- and post-Session contractile RFD relative to body mass (N/s/kg) for Session 1 (A) and Session 2 (B) for weightlifters (WL) and resistance-trained (RT) adults. Contractile RFD was calculated at in time intervals 0-30, 50, 100, 200, and 400 ms from the onset of contraction. The peak RFD was determined from within the entire contraction period. *Significant difference within Session between groups (P < 0.05); ‡Significant difference within Session for group (P < 0.05); §Significant difference between Sessions at the same time from contraction onset for group (P < 0.05).
5.4.4 Contractile Impulse

Contractile impulse was greater in weightlifters than in resistance-trained adults at 0-400 ms ($P < 0.05$). A group x time interaction was also evident 0-400 ms ($P < 0.05$) (Figure 5-5). Post-hoc paired comparisons showed that pre-Session 1 contractile impulse was 45.6 ± 6.8% higher in weightlifters when compared to resistance-trained adults at 0-400 ms ($P = < 0.001$; $d = 0.97$). No significant within- or between-session differences in CI were observed for weightlifters. Conversely, CI in the resistance-trained adults pre-Session 2 was higher than the respective pre-Session 1 value at 0-400 ms ($P < 0.05$; $d = 0.49$).

![Figure 5-5](image_url)

**Figure 5-5**: Mean (SE) pre- and post-Session contractile impulse relative to body mass (N/s/kg) for Session 1 (A) and Session 2 (B) for weightlifters (WL) and resistance-trained (RT) adults. Contractile impulse was determined as the area under the force-time curve at time intervals 0-30, 50, 100, 200, and 400 ms from the onset of contraction. *Significant difference within Session between groups ($P < 0.05$); §Significant difference between Sessions at the same time from contraction onset for group ($P < 0.05$).
5.4.5 Muscle Architecture

The VL $\theta_p$ of the weightlifters was greater than that of the resistance-trained adults ($P = < 0.05$) (Figure 5-6), whereas no significant difference in anatomical ($P = 0.59$) or physiological ($P = 0.47$) muscle thickness existed between the groups (Table 5-2). The pre- and post-Session 2 $\theta_p$ were $19.5\%$ ($P < 0.05; d = 0.85$) and $22.5\%$ ($P < 0.05; d = 0.88$) greater, respectively, in weightlifters compared to the corresponding values in resistance-trained adults. No differences in VL $\theta_p$ existed between the groups within-Session 1 (pre-Session: $P = 0.20; d = 0.58$, post-Session: $P = 0.09; d = 0.50$) (Figure 5-6). Contrary to our expectations, there was no effect of time on $\theta_p$ ($P = 0.58$) or muscle thickness ($P = 0.11$).

No associations existed between pre-Session PF and VL $\theta_p$ in weightlifters (Session 1: $r = 0.08; P = 0.76$. Session 2: $r = -0.01; P = 0.97$) or resistance-trained adults (Session 1: $r = -0.13; P = 0.64$. Session 2: $r = -0.16; P = 0.57$). In addition, the relative within-Session changes in PF, contractile RFD, relRFD and CI were not significantly correlated with the corresponding relative changes in $\theta_p$ or muscle thickness for either group.

**Figure 5-6**: Mean (SE) pre- and post-Session vastus lateralis pennation angle in weightlifters (♦) and resistance trained adults (■) for two high intensity resistance exercise sessions performed on the same day. *Significant difference between groups at the same time point ($P < 0.05$).
Table 5-2: Mean (± SE) muscle thickness of the vastus lateralis obtained from sagittal plane ultrasound images in weightlifters (WL) and resistance-trained adults (RT) across two high-intensity resistance exercise sessions performed on the same day.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Session 1</th>
<th></th>
<th>Session 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Anatomical Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>WL</td>
<td>22.5 ± 0.9</td>
<td>23.0 ± 0.9</td>
<td>23.3 ± 1.0</td>
<td>23.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>22.9 ± 0.8</td>
<td>22.9 ± 0.8</td>
<td>22.2 ± 0.9</td>
<td>22.7 ± 0.7</td>
</tr>
<tr>
<td>Physiological Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>WL</td>
<td>23.3 ± 0.9</td>
<td>23.7 ± 0.9</td>
<td>24.3 ± 1.1</td>
<td>24.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>23.5 ± 0.8</td>
<td>23.5 ± 0.8</td>
<td>22.8 ± 0.9</td>
<td>23.3 ± 0.7</td>
</tr>
</tbody>
</table>
5.5 Discussion

The major findings of this study were: 1) weightlifters demonstrated a greater ability to generate and sustain PF during repeated HIRE bouts of the same muscle group; 2) weightlifters demonstrated a greater ability to rapidly generate force in the later stages of muscle contraction (i.e. ≥100 ms) regardless of their PF, 3) despite exhibiting within-session and between-session decreases in PF, contractile RFD, relRFD and CI were maintained and/or enhanced between-sessions in resistance-trained adults, and, 4) the relative changes, or lack thereof, in PF, contractile RFD, relRFD and CI for weightlifters and resistance-trained adults were not associated with relative changes in VL $\theta_p$ or muscle thickness for either group.

To the best of our knowledge, this is the first study to demonstrate divergent muscle functional and architectural responses of competitive weightlifters and resistance-trained adults to two equivalent HIRE sessions performed on the same day. Our findings of sustained and/or enhanced contractile RFD, and CI between successive HIRE sessions extends previous observations that such training does not compromise the ability to rapidly generate force in competitive weightlifters and resistance-trained adults (Chiu et al., 2004; Häkkinen et al., 1988a; 1992).

Of particular interest in this study was the lack of association between PF, contractile RFD, relRFD, CI and skeletal muscle architecture. The VL $\theta_p$ of the resistance-trained group were consistent with values (similarly obtained with the knee at 45°) reported in recreationally active adults who took part in a five-week resistance exercise program (Blazevich et al., 2007). However, despite PF being 26-38% greater in weightlifters when compared to resistance-trained adults, no significant association existed between pre-Session PF and the larger $\theta_p$ exhibited by these athletes. Furthermore, the lack of change in VL $\theta_p$ over time is in contrast to previous evidence which suggests that the contraction history of a muscle can influence the post-contraction $\theta_p$ (Csapo et al., 2011; Kubo et al., 2001; Mahlfeld et al., 2004). However, the different exercise modes, intensities, volumes and muscle contraction types used in each investigation may partly account for this discrepancy. Following exhaustive dynamic and repeated isometric contractions, acute increases in $\theta_p$ and muscle thickness have been attributed to increased tendon compliance and muscle perfusion (Brancaccio et al., 2008; Csapo et al., 2011; Kubo et al., 2001). However, the high volume of
repeated repetitions used in these investigations contrasts the relatively low volume, intermittent nature of our exercise protocol.

In addition to influencing skeletal muscle architecture, the compliance of tendon and elastic filaments also affects PF and contractile RFD (Bojsen-Møller et al., 2005). In response to active stretch, skeletal muscle stiffness increases via a $\text{Ca}^{2+}$ dependent potentiation of elastic filaments (Bagni et al., 2004; Cormie et al., 2011a; Joumaa et al., 2008; Rassier et al., 2002). Although a stiffer series elastic component promotes smaller changes in $\theta_p$ (Pearson et al., 2005), it has been associated with enhanced force generation (Joumaa et al., 2008; Rassier et al., 2002). Furthermore, previous research has shown that the stiffness of the tendon-aponeurosis complex may account for $\sim$30% of the variance in contractile RFD (Bojsen-Møller et al., 2005). Therefore, it is possible that such a mechanism enabled weightlifters and resistance-trained adults to sustain contractile RFD and CI despite exhibiting no significant within or between session changes in muscle architecture. In addition to the influence of mechanical stimuli, increases in tendon compliance and $\theta_p$ have been shown to occur in the evening, relative to the morning (Pearson et al., 2005). However, in the current investigation there is little evidence of such diurnal variation as no significant within- or between-session changes in VL $\theta_p$ occurred for either group. As no associations existed between the relative changes in muscle function and the relative changes, or lack thereof, in muscle architecture, other physiological factors must be responsible for the divergent responses in PF, contractile RFD and CI between weightlifters and resistance-trained adults.

Contractile RFD during the early phase of muscle contraction (0-50 ms) is related to the intrinsic contractile properties of skeletal muscle (Aagaard et al., 2002). Conversely, maximal strength has been shown to account for approximately 80% of the variance in contractile RFD during the later stages (i.e. 100-250 ms) of muscle contraction in sedentary adults (Andersen et al., 2006). In the current investigation, PF was not the underlying mechanism responsible for the divergent contractile RFD responses between groups pre-Session 1 as relRFD (at $\geq$100 ms) remained significantly higher in weightlifters when compared to resistance-trained adults. However, the significant within- and between-session increases in relRFD in the resistance-trained adults occurred as a function of their decreased ability to sustain PF and their ability to maintain and/or increase contractile RFD.
Temporal changes in contractile RFD and CI have been attributed to alterations in motor unit recruitment, firing frequency and synchronization (Aagaard et al., 2002; Mellor et al., 2005; Semmler, 2002; Thorlund et al., 2008). Motor unit synchronization is a proposed training-induced adaptation of the nervous system that enables the co-activation of numerous muscle groups to effectively enhance force generation (Aagaard et al., 2002; Mellor et al., 2005; Semmler, 2002). As contractile RFD and CI are paramount to weightlifting performance (Garhammer et al., 1992; Schilling et al., 2008), it is likely that training-induced improvements in inter-muscular coordination allow weightlifters to maintain or enhance these variables during repeated dynamic exercise sessions. Although both experimental groups were familiar with the dynamic barbell front squat, we cannot discount the possibility that the weightlifters demonstrated a more effective recruitment of agonist and synergistic muscle groups, and a decreased co-contraction of antagonists, during the performance of the dynamic exercise sessions (Cormie et al., 2011a; Mellor et al., 2005; Milner-Brown et al., 1975). However, since muscular function was assessed using an isometric test (relevant to the dynamic exercise of interest), the influence of potential differences in inter-muscular coordination between groups is likely to have been minimized.

Finally, as isometric PF is known to increase over the time course of a day (Gauthier et al., 1996; Martin et al., 1999; Tamm et al., 2009), such diurnal variation may partly account for the ability of weightlifters to sustain PF. However, this time of day effect may have also masked the full extent of the resistance-trained group’s decline in PF over the two HIRE sessions. Our inability to account for the influence of diurnal variation on muscle function and tendon compliance is therefore a limitation of our study.
5.6 Conclusion

As hypothesized, competitive weightlifters demonstrated a greater ability to sustain PF across repeated HIRE bouts of the same muscle group when compared to resistance-trained adults. However, both highly-trained weightlifters and resistance-trained adults sustained or increased their contractile RFD and CI in response to such training. Finally, the maintenance or enhancement of muscular function was not associated with acute changes in muscle architecture for either group. These findings have important practical implications for the prescription of HIRE programs intended to improve muscular strength and power. For example, due to the inability of resistance-trained adults to maintain PF across two successive HIRE, these individuals may benefit more from performing a single session of high-intensity strength training within a given day. However, due to their ability to maintain or improve contractile RFD and CI, it would be possible to perform a subsequent low- to moderate-intensity plyometric/speed training session within the same day. Further research in this area may enable a more precise application of training loads and intensities based upon an individual’s muscle functional responses across multiple daily sessions.
6.0 PERIPHERAL BLOOD MONONUCLEAR CELL AND SKELETAL MUSCLE GENE EXPRESSION RESPONSES TO HIGH-POWER, HIGH-INTENSITY RESISTANCE EXERCISE AND FEEDING DURING RECOVERY
6.1 Abstract

Mechanical stimuli and nutrient availability influence many of the acute responses and adaptations to resistance exercise. The purpose of this investigation was to examine whether the influence of high-power, high-intensity resistance exercise (HIRE), similar to that performed by competitive weightlifters, would induce the expression of the chemokine/receptor pairings SDF-1/CXCR4 and MCP-1/CCR2 in peripheral blood mononuclear cells (PBMCs) and skeletal muscle. Furthermore, the effect of a post-exercise meal on the expression of the identified chemokine/receptor pairings during post-exercise recovery was also investigated. Seven resistance-trained men took part in three experimental conditions; Trial A – Exercise and Meal, Trial B – Exercise and No Meal and, Trial C – No Exercise and No Meal. The mRNA responses of CXCR4 and CCR2 in PBMCs were determined at pre- and 3 h post-exercise under each condition. Changes in serum glucose, creatine kinase (CK) and cortisol levels were measured from pre- to 3 h post-exercise. Finally, the mRNA levels of SDF-1 and the mRNA and protein levels of MCP-1 were determined in skeletal muscle (3 h post-exercise). High-power, HIRE resulted in a 6.8-fold increase in MCP-1 mRNA in skeletal muscle and a 2.5-fold increase in CCR2 mRNA in PBMCs (P < 0.05). These exercise-induced transcriptional responses were attenuated by a post-exercise meal. The abundance of MCP-1 protein in skeletal muscle increased following exercise, but was unaffected by the post-exercise meal. The CK levels suggested that a modest degree of muscle damage occurred in response to the exercise. However, in response to post-exercise feeding, the lower transcriptional response of MCP-1 and CCR2 in muscle and PBMCs, respectively, suggests a nutrient-regulatory effect during the recovery from high-power, HIRE. In contrast, the SDF-1/CXCR4 pairing was not responsive to either exercise trial. In conclusion, a post-exercise meal attenuated the exercise-induced expression of the MCP-1/CCR2 pairing which is an important modulator of muscle repair and regeneration following injury. Although such findings suggest that the absence of a post-exercise meal enhances the potential for muscle repair and regeneration, via an increase in MCP-1 and CCR2 gene expression, further research is required to determine the functional relevance of this exercise-nutrient interaction on athletic performance.
Muscular strength and power is paramount to the successful performance of many athletic activities. Consequently, a great deal of research has examined the efficacy of various training methods designed to improve strength and power output and its transference to athletic performance (Baker et al., 1999; Cormie et al., 2011b; Cronin et al., 2005; Harris et al., 2000; Hori et al., 2005; Kawamori et al., 2005; Stone et al., 2003a; 2005; 2006a). In addition, there is growing evidence of the acute neuromuscular and neuroendocrine responses to strength and power resistance exercise in trained participants (Cormie et al., 2011a; Häkkinen, 1989b; 1990; Kraemer et al., 1999; 2003; Linnamo et al., 2005; McCaulley et al., 2009). However, to date, very few investigations have examined the acute genomic responses following high-power, high-intensity resistance exercise (HIRE), similar to that performed by competitive strength and power athletes (Lamas et al., 2010; Lehti et al., 2009). As competitive weightlifters endure uniquely intensive training structures that involve multiple daily HIRE sessions and repeated overload and recovery periods, such research will help to explain how these athletes are able to recover from and adapt to such extreme workloads.

The results of Study 1 showed that high-power, HIRE resulted in a robust exercise-induced transcriptional response of CXCR4 in the PBMCs of highly-trained competitive weightlifters after overload training. CXCR4 is one of the most studied chemokine receptors due to its involvement in the regulation and migration of PBMCs and various stem/progenitor cells during inflammatory conditions (Bleul et al., 1996; Busillo et al., 2007; Miller et al., 2008; Nagatomi et al., 2002; Okutsu et al., 2005). Despite CXCR4’s diverse expression, it selectively binds to one ligand, SDF-1 (Busillo et al., 2007). In response to tissue injury, SDF-1 expression has been shown to increase within the area of damaged tissue (Kucia et al., 2004b; Ratajczak et al., 2006). The resulting SDF-1 chemoattraction gradient draws circulating CXCR4+ cells to the site of damage, thereby enhancing the potential for tissue repair and regeneration (Kucia et al., 2004b; Ratajczak et al., 2006). In light of SDF-1/CXCR4’s established roles in the repair and regeneration of skeletal muscle, and the novel exercise-induced response of CXCR4 found in Study 1, investigation of the expression of SDF-1 in muscle following exercise similar to that performed by competitive weightlifters was undertaken. Furthermore, as previous investigations (refer to Section 2.8.1) have demonstrated that aerobic exercise-induced increases in cortisol up-regulate the expression of CXCR4 on T-lymphocytes in a dose-dependent manner (Nagatomi et al., 2002; Okutsu et al.,
2002; Okutsu et al., 2005), the influence of resistance-exercise induced increases in cortisol on the expression of CXCR4 mRNA in PBMCs was also investigated.

Study 1 also demonstrated that high-power, HIRE has the potential to elicit a significant degree of muscle damage, coincident with decrements in performance and a robust immunological and inflammatory stress response in highly-trained weightlifters. Thus, other early responsive inflammatory cytokine and/or chemokine receptor pairings that have known links to muscle repair and regeneration pathways were also considered for this investigation. Following traumatic injury or exercise-induced muscle damage, Monocyte Chemotactic Protein-1 (MCP-1) is increased within areas of damaged tissue and within the circulation (Chazaud et al., 2003; Cholewicki et al., 1991; Paulsen et al., 2005; Peake et al., 2005b; Summan et al., 2003a, b; Yahiaoui et al., 2008). In response to elevated MCP-1 levels, circulating PBMCs that are expressing the associated CCR2 receptor are attracted to areas of tissue damage to aid with phagocytosis and subsequent repair (Fantuzzi et al., 1999; Loetscher et al., 1994; Uguccioni et al., 1995). As such, previous investigations into damaging exercise protocols, that have also resulted in decreased muscular performance, have demonstrated increased leukocyte infiltration within skeletal muscle (Fielding et al., 1993; MacIntyre et al., 1996). In addition to exercise-induced muscle damage, exercise intensity and circulating cortisol have been shown to be positively associated with plasma concentrations of MCP-1 and the expression of CCR2 on monocytes (refer to Section 2.8.4), respectively, during aerobic exercise (Okutsu et al., 2008; Peake et al., 2005b). However, the influence of resistance-exercise induced increases in cortisol on the expression of CCR2 in PBMCs has yet to be examined.

There is a growing body of evidence demonstrating that MCP-1 also plays an important role in determining the adaptive responses to exercise (Harmon et al., 2010). For example, eight genetic variants in the MCP-1 and CCR2 genes have been associated with initial measures of skeletal muscle strength and the strength response to resistance exercise in healthy, untrained men and women (Harmon et al., 2010). From these previous findings it is evident that the MCP-1/CCR2 pairing not only plays a critical role in muscle repair and regeneration following injury, but is also an important regulator of adaptation to resistance exercise in untrained adults. However, the responsiveness of MCP-1 (in muscle) and CCR2 (in PBMCs) in highly-trained adults following high-power, HIRE that has the potential to elicit muscle damage, has yet to be examined.
Lastly, nutrient availability is a potent modulator of many of the acute responses to resistance exercise that lead to chronic adaptations in muscle (Braun et al., 2004; Hargreaves et al., 2002; Hawley et al., 2006; 2007; 2010; Roberts et al., 2010). For example, during Study 1, a 2 wk overload period in competitive weightlifters resulted in a significant exercise-induced increase in the expression of a stress-responsive negative regulator of the mTOR pathway; DDIT4. The increased expression of DDIT mRNA in PBMCs coincided with negative changes in the self-reported diet and body weight of the competitive weightlifters. These results were in accordance with previous evidence that DDIT4 is upregulated during periods of energy stress and starvation. At present, the majority of research regarding the acute (i.e. 0–6 h post-exercise) genomic responses to resistance exercise has been conducted with untrained individuals in an unfed state (i.e. no pre- or post-exercise meal) (Buford et al., 2009; Mascher et al., 2008; Pilegaard et al., 2000; Raue et al., 2006; Willoughby et al., 2002). However, as previous research has demonstrated that acute and short-term periods of energy restriction result in distinctive genomic signatures (Bouwens et al., 2007; Crujeiras et al., 2008; Hulmi et al., 2009), such findings may not truly reflect the transcriptional responses arising from the exercise stimulus alone. Furthermore, resistance-trained adults and competitive athletes are encouraged to consume both pre- and post-exercise meals to help ensure that the prescribed training intensities and volumes are met and to support subsequent recovery processes (Burke et al., 2004; Levenhagen et al., 2001; Maughan et al., 2002; Rodriguez et al., 2009b). Thus, performing HIRE in an unfed state is not ecologically valid in the context of these populations. Therefore, the results obtained from such studies do not necessarily reflect the acute responses that lead to enhanced athletic performance in well-trained individuals. Although feeding following intense resistance exercise is known to augment protein and glycogen synthesis in skeletal muscle, the influence of a post-exercise meal on the acute chemokine responses to high-power, HIRE in well-trained adults has yet to be elucidated. Such research will enable a greater understanding of how a modifiable factor such as feeding can influence various muscle repair and regeneration pathways following intense resistance exercise.

Thus, the aims of this investigation were to: (1) determine the effects of an acute bout of high-power, HIRE on the mRNA levels of SDF-1 and the mRNA and protein levels of MCP-1 in skeletal muscle; (2) quantify the PBMC transcriptional response of the associated chemokine receptors, CXCR4 and CCR2, following high-power, HIRE; (3) determine the effects of a post-exercise meal on the transcriptional responses of SDF-1/CXCR4, MCP-
1/CCR2 and the protein expression levels of MCP-1 (in muscle); and (4) determine if CXCR4 and CCR2 mRNA expression in PBMCs was associated with exercise-induced increases in circulating cortisol following high-power, HIRE.

It was hypothesized that: (1) high-power, HIRE would elicit an increase in SDF-1 mRNA and MCP-1 mRNA and protein expression within skeletal muscle; (2) CXCR4 and CCR2 mRNA expression would increase in circulating PBMCs in response to the exercise protocol; (3) post-exercise feeding would attenuate the exercise-induced mRNA and protein expression responses of SDF/CXCR4 and MCP-1/CCR2 in skeletal muscle and PBMCs; and (4) a positive association would exist between exercise-induced increases in circulating cortisol and CXCR4 and CCR2 mRNA expression in PBMCs.
6.3 Methods

6.3.1 Participants

Seven healthy resistance trained men (Table 6-2) volunteered to participate in this repeated-measures within-subject design study. They were recruited via Participant Recruitment Flyers posted at local gyms, on University notice boards and University of Auckland department websites (refer to Appendix B). As a requirement of the study, all participants were: 1) males aged between 18-35 years; 2) free from acute or chronic injury at the time of data collection; 3) not using any performance enhancing supplements (e.g. creatine) or banned substances (WADA, 2011); and 4) had performed resistance exercise two or more times per week for the past 6 months. According to the Pre-participation Questionnaire (refer to Appendix B), participants had performed 3-5 resistance exercise sessions per week for the last 6 months and had been performing resistance exercise on a regular basis for $5.7 \pm 2.3$ years. Although all participants had a history of sport participation, none were competing at the time of data collection. Each participant provided written, informed consent prior to commencing the investigation. This study was approved by the University of Auckland Human Participant Ethics Committee (refer to Appendix B).

6.3.2 Experimental Design

To determine the influence of high-power, HIRE and post-exercise feeding on the inflammatory chemokine/receptor pairings SDF-1/CXCR4 and MCP-1/CCR2, all participants completed three experimental conditions: Trial A – Exercise and Meal; Trial B – Exercise and No Meal; and, Trial C – No Exercise and No Meal (rest only control condition).

All participants took part in a familiarisation session at least three days prior to commencing the first experimental trial. During the familiarisation session, body composition was measured and the following tests were conducted: 1) maximum isometric strength; 2) maximum isometric rate of force development; 3) dynamic muscular strength; 4) muscular power; and, 5) high-power resistance exercise ability (refer to section 6.3.3 for detailed procedures).
Participants were instructed to record their diet in detail for the two days prior to the first experimental trial on the Nutrition Diary sheets provided (refer to Appendix C). Participants were told that they would be required to replicate the same diet during the two days prior to the remaining experimental trials and Nutrition Diary sheets were also completed on these occasions. A dietary assessment of the two-day Nutrition Diaries was conducted using Foodworks Professional (New Zealand Version 6.0).

On the morning of each experimental trial, participants reported to the University of Auckland’s Exercise Physiology Laboratory between 7:00am and 9:00am following an overnight fast (≥8 h). Each participant began each trial condition at the same time of day (± 30 min of the previous trial). Three participants performed the Exercise and Meal trial first and four participants performed Exercise and No Meal trial first. The No Exercise and No Meal control condition was the second trial performed for all participants. Between trials, participants recommenced their habitual training but were instructed to refrain from all exercise for 48 h prior to the subsequent trial. In addition, all participants were required to abstain from caffeine and alcohol for at least 24 h prior to all trial conditions. After providing resting and fasted (overnight fast ≥ 8 h) baseline blood samples, participants consumed a standardized 3,251 kJ meal consisting of 103 g carbohydrate (54.6% of nutrient energy yield), 34 g protein (19.0%), and 22 g fat (26.4%) (Appendix B). Pre-exercise blood samples were obtained 1.5 h after the meal and immediately prior to the 90 min exercise protocol (and 90 min prior to the rest only period for the control condition). Post-exercise blood samples were obtained immediately post-exercise (0 min) and 3 h post-exercise. During the Exercise and Meal trial only, the same standardized meal was provided after the immediate post-exercise blood sample was obtained. Water was consumed ad libitum throughout the trials. During all trials, a 3 h post-exercise muscle biopsy was obtained from the participant’s vastus lateralis. A non-exercise control condition was chosen over the use of a pre-exercise muscle biopsy as it was believed that a pre-exercise biopsy would have affected the participant’s ability to complete the prescribed exercise session. A similar protocol has been used in previous investigations of the acute molecular responses to high-power resistance exercise (Lehti et al., 2009) and aerobic exercise in the fasted and fed states (Harber et al., 2010).
Figure 6-1: Overview of the three experimental trial conditions.

**Trial A**

*Exercise + Meal*

**Trial B**

*Exercise + No Meal*

**Trial C**

*No Exercise + No Meal*

**All Trials**

- Breakfast
- Blood Sample
- Biopsy
6.3.3 Familiarisation Session

Participants were instructed to refrain from exercise for at least 24 h prior to the familiarisation session and arrived at the University of Auckland Training centre in an approximately two-hour post-prandial state.

6.3.3.1 Anthropometric Measurements

All anthropometric measurements were taken on the participant’s right side in accordance with the standard specifications of the International Society for the Advancement of Kinanthropometry (Appendix B). Nine skin-fold sites (chest, bicep, triceps, subscapular, abdomen, suprailiac, supraspinale, calf and thigh) were measured using Harpenden skin-fold calipers (Baty International, England). The mean of two measurements (mm) were used for each skin-fold site. The body density of each participant was estimated by the method of Jackson et al. (1978) and the resulting value was used to estimate the percentage body fat of each participant (Siri, 1961) (refer to Appendix B). In addition, the sum of the nine skin-fold measurements (mm) was calculated to allow for comparisons between participants.

6.3.3.2 Isometric Strength Testing

Maximal isometric peak force (PF) and contractile rate of force development (RFD) were measured during a maximal voluntary isometric contraction in a front squat position as per the methods in Study 2 (Section 5.3.4).

6.3.3.3 Dynamic Strength Testing

Each participant’s one-repetition maximum (1RM) barbell back squat was determined as a measure of dynamic muscular strength. The back squat was performed in a squat rack fitted with safety bars. Participants used a shoulder width stance and were required to descend to a parallel depth (i.e. the back of the thigh at the gluteal fold descends below the posterior aspect of the knee) (Chiu et al., 2004). The depth of each squat was confirmed visually by the investigator. Furthermore, a set of elastic bands were set at the correct depth behind the participant to provide kinesthetic feedback regarding the squat depth. Participants performed
a warm up set consisting of ten dynamic back squats to a parallel depth using 50% of their recently attained 1RM. Following a 1 min rest period, the load of the barbell was increased to ~70% 1RM and the participant performed three repetitions at this sub-maximal load. A series of single repetition attempts of increasing load followed and the highest weight achieved (kg) prior to failure was recorded as the participant’s 1RM. A rest interval of 1-3 min was taken between attempts.

6.3.3.4 Vertical Jump Testing

The maximal vertical jump test is a reliable measure of muscular power (Harris et al., 2000; Malisoux et al., 2006). In addition, strong correlations have been demonstrated between vertical jump performance and weightlifting ability (Carlock et al., 2004; Fry et al., 2006a).

Following a standardized warm up consisting of light jogging and calisthenics, each participant performed three maximal static (SJ) and three counter-movement vertical jumps (CMJ) on a calibrated 59.5 x 89.5 cm force plate (Kelba Onspot Power Plate, Sydney, Australia). A 30 sec rest interval was taken between subsequent attempts and a 2 min rest was taken between the static and counter-movement jump trials. The static jumps were performed from a parallel thigh position (concentric muscle action only). Conversely, participants began the counter-movement jumps in a standing position, rapidly descended to a required parallel thigh position and immediately performed an “explosive” upward movement. Each jump trial produced a vertical force-time curve from which “flight-time” (denoted as $t_{air}$) was calculated as the change in time between the takeoff and impact (refer to Appendix H). All data was sampled at 1,000 Hz (Chart™ V5.5.5, AD Instruments) with a low-pass digital filter applied with a cut-off frequency set at 20 Hz. The maximum jump height (h), defined as the maximum rise in the centre of gravity, was calculated from the vertical velocity ($V_v$) at takeoff (Bosco et al., 1979) (refer to Appendix H).
6.3.3.5 High-Power Resistance Exercise Testing

Due to the complexity of the power snatch (hang) and power clean/front squat/push press complex (refer to Appendix E for a description of each exercise), a trained exercise professional monitored each lift to ensure correct technique was used.

The power snatch (hang) and the power clean/front squat/push press complex were chosen on the basis that these exercises: 1) require less specialized training in order to execute; 2) posses strong kinematic similarities with the competitive snatch and clean and jerk; and 3) are associated with high-power outputs (Cormie et al., 2007; Winchester et al., 2005; 2009).

After performing a standardized warm up using a 20 kg barbell, participants were required to reach a 1 repetition maximum (1RM) for each exercise within 6-8 sets. The highest weight achieved (kg) prior to failure was recorded to calculate the loads to be used during the subsequent exercise trials. A rest interval of 1-3 min was taken between attempts.

6.3.4 High-Power, High-Intensity Resistance Exercise Session

Following a standardized warm up consisting of light jogging and calisthenics, participants performed the six exercises in Table 6-1. The exercises were selected based on their frequent use in conditioning programs of strength and power athletes (including weightlifters). In addition, these exercises have featured in a number of studies investigating the acute neuromuscular, hormonal and molecular responses to high-power resistance exercise (Atamaniuk et al., 2010; Häkkinen et al., 1985a; 1988a; 1989a; 1989b; 1990; Malisoux et al., 2006; Potteiger et al., 1995; Viru et al., 2003). The number of sets and repetitions and the corresponding exercise intensities (percentage of 1RM) of the power snatch (hang), power clean/front squat/push press complex and back squat were similar to that prescribed in the conditioning programs of strength and power athletes (Cormie et al., 2011b; Häkkinen et al., 1988a; Hoffman et al., 2004; Kilduff et al., 2007). A full description of the exercise intensities during each set can be found Section 6.4.3. The training volumes (sets × repetitions), exercise intensities and inter-set rest period durations were replicated during both exercise conditions (Exercise and Meal; Exercise and No Meal). At the completion of each
exercise session, participants were asked to rate the overall intensity of the session using a standard 6 – 20 Rating of Perceived Exertion (RPE) scale (Borg, 1982) (refer Appendix C).

**Table 6-1: Overview of the high-power, high-intensity resistance exercise session.**

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Sets</th>
<th>Total Repetitions per Exercise</th>
<th>Intensity (%1RM)</th>
<th>Inter-Set Rest Interval (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power Snatch (Hang)</td>
<td>7</td>
<td>16</td>
<td>55 to ~96</td>
<td>1-3.5</td>
</tr>
<tr>
<td>Power Clean/Front Squat/Push Press</td>
<td>7</td>
<td>15</td>
<td>55 to 90</td>
<td>1-3.5</td>
</tr>
<tr>
<td>Back Squat</td>
<td>6</td>
<td>12</td>
<td>70 to ~93</td>
<td>1-3.5</td>
</tr>
<tr>
<td>Box Jumps</td>
<td>3</td>
<td>9 jumps</td>
<td>Max. Speed</td>
<td>1-1.5</td>
</tr>
<tr>
<td>Hurdle Jumps</td>
<td>4</td>
<td>48 jumps</td>
<td>Max. Speed</td>
<td>1-1.5</td>
</tr>
<tr>
<td>Single-leg Triple Jump</td>
<td>4</td>
<td>32 jumps</td>
<td>Max. Speed</td>
<td>1-3.0</td>
</tr>
</tbody>
</table>

### 6.3.5 Blood Sampling and Processing

During the two exercise trials, peripheral blood samples were drawn by standard venepuncture into two 4 mL BD Vacutainer™ tubes (1 x Serum Tube and 1 x EDTA Plasma Tube - Becton Dickinson, Heidelberg, Germany) at four time points; baseline (fasted), pre-exercise (90 min after a standardized pre-exercise meal), immediately post-exercise and 3 h post-exercise. An additional 16 mL of blood was collected into two 8 mL BD Vacutainer™ CPT™ Sodium Citrate Tubes (Becton Dickinson, Heidelberg, Germany), for the isolation of peripheral blood mononuclear cells (PMBCs), at baseline and 3 h post-exercise. During the No Exercise and No Meal trial, all blood samples, as described above, were collected via standard cannulation technique.

Plasma tubes were stored on wet ice while serum tubes were left to coagulate at room temperature for 30 min prior to centrifugation. The plasma and serum tubes were then
centrifuged at 3,000 rpm for 10 min at room temperature. The upper layers of plasma and serum were carefully aspirated, aliquoted and frozen at -80°C until analysed. The blood samples obtained in the BD CPT tubes were processed within 15 min of acquisition using the PBMC isolation protocol described in Study 1 (refer Section 4.3.5).

6.3.6 Muscle Tissue Collection

During all trials, a 3 h post-exercise muscle biopsy sample was obtained from the participant’s vastus lateralis. Biopsies for the two exercise trials (Trial A and B) were obtained from separate incisions (≥ 1 cm apart) on the same leg whilst the rest only control biopsy was taken from the opposite leg.

Each procedure was performed under local anesthetic by a qualified physician trained in the Bergström percutaneous needle biopsy technique (Bergström, 1962, 1975). The skin overlying the vastus lateralis was shaved on the day to minimize the risk of surgical site infection (Kjønniksen et al., 2002). The participant was then asked to “contract” their quadriceps and the physician marked the target site (mid portion of the thigh, 15-20 cm proximal to the mid-patella). After cleaning the target site, the overlying skin was numbed by injecting up to 5 mL of local anesthetic (2% lidocaine) using a 25 G needle. After ~1-2 min an additional injection of up to 5 ml of 2% lidocaine was administered to the underlying muscle fascia (not to the muscle belly). Once the area was anaesthetized, a small incision was made (~1cm) with a surgical scalpel through the skin and underlying fascia. A 5 or 6 mm Bergström biopsy needle was then inserted through the incision into the muscle belly. The physician’s assistant manually applied suction to the needle by pulling on the attached syringe apparatus (Hennessey et al., 1997) as the trocar (biopsy needle blade) was moved in and out of the biopsy needle (refer to Figure 6-2). The suction was halted as the physician rotated the needle 90° in preparation for a second cut. Up to four cuts in total were made without removing the biopsy needle from the incision site. The resulting samples (mean weight of 144.4 ± 66.9 mg) were immediately blotted, freed of any non-muscle matter, weighed, placed into RNase free tubes and snap frozen in liquid nitrogen. Samples were stored at -80°C until analysis.
Figure 6-2: Bergström muscle biopsy needle and suction apparatus. The cutting trocar (A) is inserted into the outer needle that has a cutting chamber at the base (B). A clearing rod (C) can be inserted into the trocar to remove the biopsy sample. Suction is manually applied via a 10-50 mL syringe connected to the Bergström needle by a sterile cannula connection piece (D). Photographs are adapted from Hennessey et al. (1997).

6.3.7 Total RNA Isolation

Peripheral Blood Mononuclear Cells:

The isolated PBMC pellets, obtained from baseline and 3 h post-exercise samples, were suspended in 1,200 µL of TRIzol® Reagent (Invitrogen, Life Technologies, Karlsruhe, Germany) and processed as per the total RNA isolation methods described in Study 1 (refer Section 4.3.6).

Skeletal Muscle:

Total RNA was isolated from skeletal muscle samples obtained 3 h post-exercise. Briefly, 20-30 mg of frozen muscle tissue was homogenized using a rotary homogenizer (PRO200, PRO Scientific Inc., Oxford, USA) in 500 µL of TRIzol® Reagent as per the manufacturer’s recommendations (Invitrogen, Life Technologies, Karlsruhe, Germany). Following homogenization, 100 µL of chloroform was added to each sample and the tubes were shaken vigorously by hand for 15 sec prior to incubation for 3 min at room temperature. The samples were then centrifuged at 10,800 rpm for 15 min at 4°C to separate the homogenates into a
clear upper aqueous phase (exclusively containing total RNA), an interphase and a red lower organic phase (containing DNA and proteins). The aqueous phase of each sample was transferred to a fresh tube. An equal volume of 100% isopropanol was added and the samples were left to incubate for 10 min at room temperature. The samples were then centrifuged at 10,800 rpm for 10 min at 4°C to pellet the total RNA. After removing the supernatant, the pellets were washed by vortexing the samples briefly in 500 µL of 75% ethanol followed by centrifugation at 8,500 rpm for 5 min at 4°C. After repeating the wash step, the pellets were air dried for 5-10 min. The total RNA was resuspended in 20 µL of RNase-free water and incubated for 10 min at 55°C to aid with solubilisation. The extracted total RNA was aliquoted and stored at -80°C until further analysis. Total RNA quantity and quality was determined as described in Study 1 (refer Section 4.3.6).

6.3.8 Real-Time Quantitative Polymerase Chain Reaction (qPCR)

DNase Treatment and Reverse Transcription

All total RNA samples (PBMC and skeletal muscle) were DNase treated and reverse transcribed as described in Study 1 (refer Section 4.3.8).

DNase treatment was considered necessary as the Chemokine (C-X-C) motif) Receptor 4 assay (CXCR4 assay ID: Hs00607978_s1) contained primers and probes designed with a single exon (i.e. does not cross an intro/exon boundary) that would detect any contaminating DNA if present.

Real-Time Quantitative Polymerase Chain Reaction (qPCR)

The qPCR procedures and normalisation protocols used for the current investigation were performed as described in Study 1 (refer Section 4.3.8).

To quantify the expression of the target genes of interest in PBMC samples, the Chemokine (C-X-C motif) Receptor 4 (CXCR4) (Hs00237052_m1) and Chemokine (C-C motif) Receptor 2 (CCR2) (Hs00356601_m1) TaqMan® Gene Expression Assays were used. To quantify the expression of the target genes of interest in skeletal muscle samples, the Stromal
Cell-Derived Factor-1 (SDF-1) (Hs00171022_m1) and Monocyte Chemotactic Protein-1 (MCP-1) (Hs00234140_m1) TaqMan® Gene Expression Assays were used.

The qPCR C<sub>t</sub> data was exported into the qBase<sup>PLUS</sup> software analysis program (Biogazelle, NV, Belgium) to determine the fold changes in mRNA. The normalisation factors for each sample were monitored to account for variations in the amount of RNA, the efficiency of reverse transcription and the stability of the reference genes. The mean (± SD) of the normalisation factors across all experimental samples and qPCR runs was 1.1 ± 0.5, indicating a low variability between samples (Hellemans et al., 2007).

The final fold changes for mRNA expression levels at 3 h post-exercise for each sample were determined from the corresponding C<sub>t</sub> values using the 2<sup>-∆∆Ct</sup> method (Livak et al., 2001). The target gene expression in PBMCs for all trials was normalized to the baseline sample (assigned the arbitrary value of 1.0) and the geometric mean of the reference genes, Ribosomal protein, large PO (RPLPO) (Hs99999902_m1) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (Hs99999905_m1) (Vandesompele et al., 2002b). The target gene expression in skeletal muscle for the two exercise conditions (Trial A and B) was normalized to the No Exercise and No Meal control condition (assigned the arbitrary value of 1.0) and the geometric mean of the reference genes, RPLPO and GAPDH (Vandesompele et al., 2002b).

The experimental variation of C<sub>t</sub> replicates was assessed in qBase<sup>PLUS</sup> using the quality control criteria of ≤0.5 cycles between replicates for each sample/gene pairing (D’Haene et al., 2010). Replicates that exceeded the quality control criteria were excluded from further analyses. The maximum variation between the remaining replicates was <0.3 cycles.
6.3.9 Monocyte Chemotactic Protein-1 Analysis

MCP-1 protein levels were measured in skeletal muscle (via standard ELISA techniques) to add to the mRNA data in order to provide a thorough picture of the MCP-1 gene expression response to high-power, HIRE, with and without post-exercise feeding.

**Preparation of Tissue Lysates**

Frozen muscle tissue (12-25 mg) was homogenized using a rotary homogenizer (PRO200, PRO Scientific Inc., Oxford, USA) in low salt lysis buffer (1:10 sample weight (mg) to volume (µL)) on ice (refer to Appendix G, Table G-12). The tissue homogenate was immediately centrifuged at 4,400 g for 5 min at 4°C. The resulting supernatant was transferred to a fresh tube and centrifuged further at 2,500 g for 4 min at 4°C. For each sample, 20 µL of the final supernatant was diluted (1:10 vol/vol with Milli-Q H$_2$O) for quantification of total protein. The remaining supernatant was aliquoted and stored at -80°C until further analysis.

**Quantification of Total Protein**

The total protein content of each sample was determined by the Pierce BCA™ protein assay (Pierce Biotechnology, Inc., Rockford, IL), using the microplate procedure, as per the manufacturer’s recommendations. Bovine serum albumin (BSA) in Milli-Q H$_2$O was used as the standard. The resulting absorbances were read at 562 nm using an ELx808 Ultra Microplate Reader (Bio-Tek Instruments, Swedesboro, NJ, USA). The protein concentration of each sample was determined from the generated standard curve.

**ELISA Procedure.**

MCP-1 protein levels were analyzed using a commercially available Human MCP-1 ELISA kit (Invitrogen, Camarillo, CA). The manufacturer’s stated sensitivity of the kit was <5 pg/mL with an intra-assay coefficient of variation of 3.6%. The required regents were made from the components supplied within the kit (refer to Appendices G) and the manufacturer’s standard procedures were followed.
Briefly, 50 µL of the incubation buffer was added to all wells prior to adding 50 µL of each pre-diluted sample (at a concentration of 1.5 µg/µL of total protein), standard and control to each of the assigned ELISA-plate wells. Based upon the results of a preliminary test run, an additional lower range standard was included (7.8 pg/mL). All samples were analyzed in duplicate. 50 µL of the biotinylated Hu MCP-1 Biotin Conjugate was then added to each well, after which the plated was covered and left to incubate for 2 h at room temperature. Following the incubation, the plate was washed four times with the diluted wash buffer. 100 µL of Streptavidin-HRP Working Solution was then added to each well. The plate was covered and left to incubate for 30 min at room temperature. The plate wash step was repeated and 100 µL of Stabilized Chromogen was then added to each well. Following a 30 min incubation in the dark, 100 µL of stop solution was added to each well. The resulting absorbances were read at 450 nm using an ELx808 Ultra Microplate Reader (Bio-Tek Instruments, Swedesboro, NJ, USA). The concentration of MCP-1 within each sample was then calculated using the regression factors obtained from the standard curve. The mean of the duplicates were used for the final results and the values were expressed as pg of MCP-1 per mg of total protein (pg/mg).

6.3.10 Serum Glucose Analysis

Serum samples were sent to the LabPLUS medical laboratories (Auckland, New Zealand) for the determination of glucose concentration. Glucose levels were determined via an enzymatic colourimetric assay in which glucose is enzymatically converted to Gluconate-6-Phophatase and NADPH. The rate of change in NADPH levels are directly proportional to the glucose concentration and were determined at 340 nm on a Hitachi Modular system (P module, Roche). The concentration of glucose was calculated from the resulting absorbance values and expressed in mmol/L.
6.3.11 Serum Creatine Kinase Analysis

Serum samples were sent to the LabPLUS medical laboratories (Auckland, New Zealand) for the determination of creatine kinase (CK) concentrations. Creatine kinase levels were determined via a series of enzymatic reactions where the rate of NADPH formation is proportional to the CK concentration. NADPH levels were determined on a Hitachi Modular system (P module, Roche) at 340 nm. The concentration of CK within each sample was calculated from the resulting absorbance values and expressed in international units (U/L).

6.3.12 Serum Cortisol Analysis

Serum cortisol concentration was analyzed using a commercially ELISA kit (ALPCO Diagnostics, Salem, NH). The manufacturer’s stated sensitivity of the kit was 11.04 nmol/L with an inter-assay coefficient of variation of 5.0%. The required regents were made from the components supplied within the kit (refer to Appendix G) and the manufacturer’s standard procedures were followed.

**ELISA Procedure**

Prior to use, all reagents were brought to room temperature and plasma samples were thawed only once before analysis. Samples were analyzed in duplicate and 20 μL of each sample, standard and control were loaded into each of the assigned ELISA-plate wells. 100 μL of the conjugate working solution was then added to each well. The plate was covered with the adhesive strip and left to incubate on a plate shaker (set at approximately 200 rpm) for 45 min at room temperature. Following the incubation, the plate was washed three times with approximately 300 μL of diluted wash buffer per well. 150 μL of TMB substrate was then added to each well. The plate was left to incubate on a plate shaker (set at approximately 200 rpm) for 20 min at room temperature. Finally, 50 μL of stop solution was added to each well. The resulting absorbances were then read at 450 nm using an ELx808 Ultra Microplate Reader (Bio-Tek Instruments, Swedesboro, NJ, USA). The concentration of cortisol within each sample was then calculated using the regression factors obtained from the standard
curve. The mean of the duplicates were used for the final results and the values were expressed in nmol/L.

6.3.13 Data Presentation and Statistics

Data were analyzed with SigmaPlot 11.0 statistical software (Chicago, IL). Data are presented as mean ± SD with statistical significance set at $P < 0.05$. The following statistical procedures were undertaken to examine the relevant data;

Training Session

Independent samples $t$-tests were used to compare the training program variables (i.e. training load, training duration, reported RPE) between the Exercise and Meal and Exercise and No Meal trials.

Dietary Records and Biological Measurements

One way repeated-measures ANOVA were used to investigate the two-day dietary assessments, the mRNA levels of SDF-1 and the mRNA and protein levels of MCP-1 in skeletal muscle. Two factor condition (exercise trial) by time (pre-exercise, post-exercise) repeated-measures ANOVA were used to investigate CCR2 and CXCR4 mRNA expression in PBMCs and changes in plasma cortisol, serum glucose and serum creatine kinase. The analyses tested for main effects of experimental trial and time and any interaction between experimental trial and time. Where a significant difference between variables was determined by ANOVA, post-hoc paired comparisons were made using the method of Student-Newman-Keuls. Effect sizes were calculated according to the method of Cohen where $d = 0.8$ is considered a large effect, $d = 0.5$ is moderate, and $d = 0.2$ a small effect size (Cohen, 1992). Pearson product moment correlations were also used to evaluate the relative (percentage) change in cortisol and the relative (percentage) change in CXCR4 and CCR2 mRNA expression within each trial (pre- to post-exercise).
6.4 Results

6.4.1 Participants

The physical and performance characteristics of the participants are presented in Table 6-2. As a comparison, the participants’ maximum isometric voluntary force generating ability was greater than that of the resistance-trained males but was less than that of the male competitive weightlifters in Study 2 (refer to Section 5.4.2 and 5.4.3).

Table 6-2: Physical and performance characteristics of the participants (N = 7). Data are presented as mean (± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.0 ± 5.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>82.3 ± 6.3</td>
</tr>
<tr>
<td>Sum of 9 Skin Folds (mm)</td>
<td>132.8 ± 31.9</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>14.7 ± 3.8</td>
</tr>
<tr>
<td>Training Experience (years)</td>
<td>5.7 ± 2.3</td>
</tr>
<tr>
<td>Back Squat 1RM (kg)</td>
<td>139.0 ± 17.0</td>
</tr>
<tr>
<td>Power Snatch (Hang) 1RM (kg)</td>
<td>55.0 ± 7.0</td>
</tr>
<tr>
<td>Power Clean/Front Squat/Push Press 1RM (kg)</td>
<td>80.0 ± 11.0</td>
</tr>
<tr>
<td>MVIC Peak Force (N)</td>
<td>928.0 ± 268.0</td>
</tr>
<tr>
<td>MVIC Peak Force Relative to Body Mass (N/kg)</td>
<td>11.2 ± 3.0</td>
</tr>
<tr>
<td>MVIC Peak RFD (N/s)</td>
<td>4613.0 ± 1614.0</td>
</tr>
<tr>
<td>MVIC Peak RFD Relative to Body Mass (N/s)</td>
<td>56.4 ± 20.0</td>
</tr>
<tr>
<td>Static Squat Jump Peak Height of Centre of Mass (cm)</td>
<td>43.0 ± 6.0</td>
</tr>
<tr>
<td>Counter-Movement Jump Peak Height of Centre of Mass (cm)</td>
<td>47.0 ± 6.0</td>
</tr>
</tbody>
</table>

*MVIC: Maximal Voluntary Isometric Contraction*
6.4.2 Dietary Intakes

As the participants were required to replicate the same diet during the two days prior to each experimental session, no significant differences existed in the total caloric or macronutrient intakes between sessions. However, participant body mass differed significantly between Session 1 and Session 3 ($P < 0.05; d = 0.2$) (Table 6-3).

Table 6-3: Mean daily dietary records two days prior to each experimental session. Data are presented as mean (± SD). *Significant difference from Session 1 ($P < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass (kg)</td>
<td>82.7 ± 6.0</td>
<td>83.3 ± 5.8</td>
<td>83.8 ± 5.8*</td>
</tr>
<tr>
<td>Total Energy Intake (kJ)</td>
<td>13174.0 ± 33.62.0</td>
<td>11635.0 ± 2766.0</td>
<td>12433.0 ± 3912.0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>157.1 ± 29.6</td>
<td>135.3 ± 27.3</td>
<td>149.2 ± 45.5</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>21.6 ± 5.0</td>
<td>20.2 ± 4.9</td>
<td>22.1 ± 7.6</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>318.5 ± 79.3</td>
<td>341.3 ± 143.1</td>
<td>317.0 ± 138.2</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>41.8 ± 11.1</td>
<td>44.6 ± 11.0</td>
<td>42.1 ± 9.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>137.0 ± 66.5</td>
<td>110.2 ± 51.5</td>
<td>124.6 ± 65.1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>36.2 ± 11.8</td>
<td>34.5 ± 11.6</td>
<td>35.5 ± 14.3</td>
</tr>
</tbody>
</table>

6.4.3 High-Power, High-Intensity Resistance Exercise

All participants were able to complete the required number of sets and repetitions at the target training loads (kg) and target training heights/distance (m) during both exercise sessions (Trial A and Trial B) (Table 6-4). As the exercise sessions were replicated on both occasions, there were no differences in total training load or the exercise duration (Session 1: 96.3 ± 8.7 min; Session 2: 96.1 ± 10.0 min) between sessions (Table 6-4). Finally, the Rate of Perceived Exertion (RPE) did not differ between sessions (Session 1: 18.1 ± 1.3; Session 2: 17.7 ± 1.0 RPE).
Table 6-4: Overview of the high-power, high-intensity resistance exercise sessions showing the intensities (% 1RM), loads (kg) and heights/distances (m) prescribed. Data are presented as mean (± SD).

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Sets and Intensity</th>
<th>Total Volume (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%1RM Load (kg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6 7</td>
<td></td>
</tr>
<tr>
<td>Power Snatch (from hang position): Warm-up</td>
<td>55 65 75 80 85 ~90</td>
<td>648.8 (83.4)</td>
</tr>
<tr>
<td>with 3 reps using 20 kg bar</td>
<td>~95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29.6 (3.7) 35.9 (5.0) 41.1 (5.4) 43.9 (5.4) 47.5 (6.3) 50.0 (6.3) 52.3 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Power Clean/Front Squat/Push Press</td>
<td>55 65 75 85 90 90 75</td>
<td>866.9 (122.6)</td>
</tr>
<tr>
<td>%1RM Load (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.1 (7.2) 51.9 (6.9) 59.6 (8.2) 68.2 (10.1) 72.1 (9.7) 72.1 (9.7) 59.6 (8.2)</td>
<td></td>
</tr>
<tr>
<td>Back Squat</td>
<td>70 80 90 ~93 85 85</td>
<td>1401.6 (183.0)</td>
</tr>
<tr>
<td>%1RM Load (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>97.5 (11.8) 111.4 (13.6) 125.5 (15.2) 129.6 (15.4) 118.9 (14.5) 118.9 (14.5)</td>
<td></td>
</tr>
<tr>
<td>Box Jumps (with-counter): Warm-up 3 reps</td>
<td>1.0 1.0 1.0</td>
<td></td>
</tr>
<tr>
<td>at 0.80 m height</td>
<td>3 reps 3 reps 3 reps</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.0 1.0 1.0</td>
<td></td>
</tr>
<tr>
<td>Hurdle Jumps</td>
<td>0.75 0.75 0.75 0.75</td>
<td></td>
</tr>
<tr>
<td>(m)</td>
<td>12 reps 12 reps 12 reps 12 reps</td>
<td></td>
</tr>
<tr>
<td>Single-leg Triple Jump</td>
<td>20.1 (6.0) 20.1 (6.0) 20.1 (6.0) 20.1 (6.0)</td>
<td></td>
</tr>
<tr>
<td>Distance (m)</td>
<td>8 jumps 8 jumps 8 jumps 8 jumps</td>
<td></td>
</tr>
</tbody>
</table>

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6.4.4 Real-Time Quantitative Polymerase Chain Reaction (qPCR)

**CCR2 and CXCR4 Expression in PBMCs**

There was a main effect of experimental trial \((P < 0.05)\) and time \((P < 0.05)\) and an interaction between trial and time \((P < 0.05)\) for CCR2 mRNA expression in PBMCs. Post-hoc paired comparisons demonstrated that CCR2 mRNA expression was greater after the Exercise and No Meal trial when compared to after the Exercise and Meal and No Exercise and No Meal trials \((P < 0.05; d = 1.0 – 1.2)\) (Figure 6-3). No significant differences were detected for CXCR4 mRNA expression between trials.

![Graph showing expression levels](image)

**Figure 6-3:** Effect of high-power, high-intensity resistance exercise on CCR2 (black) and CXCR4 (grey) mRNA expression in peripheral blood mononuclear cells 3 hours post-exercise relative to pre-exercise. Data are presented as mean \((± SD)\). *Significant difference from pre-exercise \((P < 0.05)\); ‡Significant difference between trials \((P < 0.05)\).
**MCP-1 and SDF-1 Expression in Skeletal Muscle**

There was a main effect of experimental trial ($P < 0.05$) for skeletal muscle MCP-1 mRNA expression. Post-hoc paired comparisons demonstrated that MCP-1 mRNA expression was significantly greater after the Exercise and No Meal trial when compared to after the No Exercise and No Meal trial ($P < 0.05$; $d = 1.1$) (Figure 6-4). No significant differences were detected for SDF-1 mRNA expression between trials.

![Figure 6-4: Effect of high-power, high-intensity resistance exercise on MCP-1 (black) and SDF-1 (grey) mRNA expression in skeletal muscle 3 hours post-exercise relative to the No Exercise & No Meal control trial. Data are presented as mean (± SD). *Significant difference from No Exercise & No Meal ($P <0.05$).](image)

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6.4.5 Monocyte Chemotactic Protein-1

There was a main effect of experimental trial ($P < 0.05$) for MCP-1 protein levels in skeletal muscle. Post-hoc paired comparisons demonstrated that MCP-1 protein levels were greater after the Exercise and Meal and Exercise and No Meal trials when compared to after the No Exercise and No Meal trial ($P < 0.05$; $d = 1.4$) (Figure 6-4).

![Graph](image)

**Figure 6-5:** Effect of high-power, high-intensity resistance exercise on MCP-1 protein concentration in skeletal muscle 3 hours post-exercise. Data are presented as mean ($\pm$ SD).

*Significant difference from No Exercise & No Meal ($P < 0.05$).
6.4.6 Serum Glucose

There was a main effect of experimental trial \( (P < 0.05) \) and time \( (P < 0.05) \) for serum glucose levels. Post-hoc paired comparisons demonstrated that pre-exercise (fed state) glucose levels were lower than baseline (fasted state) and post-exercise (0 min and 3 h) levels during the Exercise and Meal trial \( (P < 0.05; d = 1.2 \text{ – } 1.4) \) (Figure 6-6). In addition, pre-exercise (fed state) glucose levels were also lower when compared to post-exercise (0 min) levels during the Exercise and No Meal trial \( (P < 0.05; d = 1.3) \). Furthermore, post-exercise (0 min) glucose levels during the Exercise and Meal trial were \( 13.8 \pm 16.9\% \) higher than the No Exercise and No Meal trial at the same time point \( (P < 0.05; d = 0.9) \).

**Figure 6-6:** Effect of exercise and feeding on serum glucose (mmol/L) during the experimental trials. During the Exercise & Meal trial, the post-exercise meal was provided immediately after the post-exercise blood samples. Data are presented as mean \( (\pm SD) \). ‡Significant difference from pre-exercise within trial \( (P < 0.05) \); *Significant difference from No Exercise & No Meal within time point \( (P < 0.05) \).
6.4.7 Serum Creatine Kinase

There was a main effect of time ($P < 0.001$) and an interaction between trial and time ($P < 0.001$) for serum CK levels. Post-hoc paired comparisons demonstrated that pre-exercise CK levels were lower than post-exercise (0 min and 3 h) levels during the two exercise trials ($P < 0.05$; $d = 0.8 – 1.3$) (Figure 6-7). In addition, post-exercise (3 h) CK levels were higher than pre-exercise and post-exercise (0 min) levels ($P < 0.05$; $d = 0.4 – 1.3$). Finally, the post-exercise (3 h) CK levels during the Exercise and Meal and Exercise and No Meal trials were $35.7 \pm 9.3\%$ and $32.7 \pm 19.4\%$ higher, respectively, when compared with the CK levels during the No Exercise and No Meal trial at the same time point ($P < 0.05$; $d = 1.0 – 1.1$).

Figure 6-7: Effect of exercise and feeding on serum creatine kinase (U/L) during the experimental trials. During the Exercise & Meal trial, the post-exercise meal was provided immediately after the post-exercise blood samples. Data are presented as mean (± SD). ‡Significant difference from pre-exercise (fed state) within trial ($P < 0.05$); §Significant difference from post-exercise (3 h) within trial ($P < 0.05$); *Significant difference from No Exercise & No Meal within time point ($P < 0.05$).
6.4.8 Serum Cortisol

There was a main effect of time \((P < 0.05)\) for serum cortisol concentrations. Post-hoc paired comparisons demonstrated that the baseline (fasted state) cortisol was greater when compared to pre-exercise (fed state) during the Exercise and No Meal trial \((P < 0.05; d = 1.1)\), post-exercise (0 min) during the No Exercise and No Meal trial \((P < 0.05; d = 1.3)\) and post-exercise (3 h) during the Exercise and Meal \((P < 0.05; d = 1.0)\) and Exercise and No Meal trials \((P < 0.05; d = 0.9)\) (Figure 6-8). Furthermore, post-exercise (0 min) cortisol concentrations during the Exercise and Meal and Exercise and No Meal trials were 24.0 ± 40.9% and 13.5 ± 56.7% higher, respectively, when compared to the same time point during the No Exercise and No Meal trial \((P < 0.05; d = 0.8 – 0.9)\). There were no associations between the relative changes in serum cortisol and the relative changes in CXCR4 and/or CCR2 mRNA expression in PBMCs.

**Figure 6-8:** Effect of exercise and feeding on serum cortisol levels (nmol/L) during the experimental trials. During the Exercise and Meal trial, the post-exercise meal was provided immediately after the post-exercise blood samples. Data are presented as mean (± SD). *Significant difference within trial from baseline \((P <0.05)\); ‡Significant difference from No Exercise & No Meal within time point \((P <0.05)\).
6.5 Discussion

The major findings of this study were that an acute bout of high-power, HIRE caused a robust increase in MCP-1 mRNA (6.8-fold) and protein in skeletal muscle and a 2.5-fold increase in the mRNA of its receptor, CCR2, in the PBMCs of resistance-trained men. In addition, a post-exercise meal attenuated the increase in MCP-1 mRNA in muscle and CCR2 mRNA in PBMCs, but had no effect on the concentration of MCP-1 protein in muscle (3 h post-exercise). In contrast, an acute bout of high-power, HIRE had no effect on the mRNA expression of SDF-1 in muscle or its associated receptor, CXCR4, in PBMCs. Furthermore, there were no associations between exercise-induced serum cortisol levels and changes in CXCR4 and/or CCR2 mRNA expression in PBMCs. Collectively, these results add to the limited data on the acute genomic responses following high-power, HIRE (Lamas et al., 2010; Lehti et al., 2009) by investigating the responsiveness of inflammatory chemokines that have known links with muscle repair and regeneration pathways.

The prescribed exercise protocol was comprised of high-intensity, whole-body weightlifting and body weight plyometric exercises. The exercise intervention was designed to mimic training elements common to competitive weightlifters and other strength and power athletes, and all exercises were performed with maximum effort (i.e. “explosively”).

Inflammatory chemokine transcripts such as MCP-1 are among the earliest changes in gene expression following exercise-induced muscle damage (Chen et al., 2003; Hubal et al., 2008; Smith et al., 2008). The CK responses seen here indicated that a modest degree of muscle damage had occurred during the exercise trials. The CK levels were comparable to those seen during the early recovery (i.e. 0-3 h post-exercise) from maximal eccentric exercise and unaccustomed resistance exercise (Lee et al., 2002; Stupka et al., 2001; Thalacker-Mercer et al., 2009). However, as the CK levels did not differ between the two identical exercise trials, it is unlikely that the difference in MCP-1/CCR2 expression between the Exercise and Meal and Exercise and No Meal trials was attributable to difference in muscle injury, but instead was due to the post-exercise meal.

The high-power, HIRE protocol resulted in a robust 6.8-fold increase in MCP-1 mRNA (3 h post-exercise) in skeletal muscle and, as hypothesized, this response was attenuated by a post-exercise meal (3.2-fold increase). These results add to previous evidence demonstrating
that altered substrate availability affects the regulatory processes governing gene expression in muscle (Arkinstall et al., 2004; Girard et al., 1997; Hargreaves et al., 2002; Jump et al., 1999; Towle et al., 1997; Tunstall et al., 2005; Vaulont et al., 2000). For example, glucose availability strongly influences the initiation of gene transcription (Girard et al., 1997; Towle et al., 1997; Vaulont et al., 2000) and thus has the potential to modulate many of the adaptive responses to exercise. Moreover, these MCP-1 mRNA responses were observed following an exercise protocol that is commonly prescribed to athletes (and resistance-trained adults) with the intention of improving muscular strength and power. Thus, the results obtained from the current investigation represent an ecologically valid exercise-induced MCP-1 mRNA response in young, healthy human skeletal muscle. To the best of our knowledge, only one previous investigation has documented the exercise-induced transcriptional response of MCP-1 in the skeletal muscle of healthy young adults following resistance exercise (Hubal et al., 2008). In response to two bouts of 300 maximal eccentric contractions (separated by 4 wk), Hubal et al. (2008) reported 9.2- and 11.8-fold increases in skeletal muscle MCP-1 mRNA levels 6 h after the first and second bout of resistance exercise, respectively. In contrast, large increases (e.g. ~32-fold) in MCP-1 mRNA levels have been documented in the later stages of recovery following extensive injury (e.g. 1-3 days post-freeze-induced injury) in mouse skeletal muscle (Warren et al., 2004). Relative to these previous findings, the lower mRNA levels reported in the current investigation are likely due to: 1) the lower severity of tissue damage that was sustained; 2) possible interspecies differences in the expression of MCP-1 (i.e. human versus mouse tissue); 3) the type of muscle contraction/s performed; 4) the training experience of the participants (i.e. resistance-trained versus untrained adults); 5) differences in the sample collection time frame (i.e. 3 h post-exercise versus 1-3 days post-exercise); and 6) a delay in post-exercise feeding (i.e. an immediate post-exercise meal versus 3 h post-exercise meal).

In contrast to the transcriptional response of MCP-1, a post-exercise meal did not attenuate the exercise-induced increases in MCP-1 protein in skeletal muscle (3 h post-exercise). Following the Exercise and Meal and Exercise and No Meals trials, MCP-1 protein levels were increased (relative to the rest only control condition) by 63.3 ± 51.7% and 88.8 ± 70.3, respectively (refer to Figure 6-5). The exercise-induced increases in MCP-1 protein expression were minor when compared with the responses seen following various injury models. For example, following femoral artery excision, Shireman et al. (2006) reported that murine MCP-1 protein levels were maximally increased in ischemic gastrocnemius tissue 3
days post-injury (85 ± 13 pg/mg). Nonetheless, because MCP-1 can easily travel between the tissue and circulation, absolute protein levels in tissue samples may not actually reflect the total amount of protein being produced (Hubal et al., 2008). Thus, the majority of investigations into the MCP-1 response to exercise have measured the circulating MCP-1 protein levels in plasma (Nieman et al., 2005b; Paulsen et al., 2005; Peake et al., 2005b).

However, the direct quantification and localization of MCP-1 within skeletal muscle following damaging exercise may provide better insight into the acute recovery and adaptation processes. For example, MCP-1 has been shown to be colocalized with resident macrophages and satellite cell populations during injury (Hubal et al., 2008). These findings suggest that satellite cells are either “primed” to produce more MCP-1 or, more MCP-1 protein is chemotactically attracted to satellite cells during tissue injury (Chazaud et al., 2003; De Rossi et al., 2000; Hubal et al., 2008; Warren et al., 2005). Furthermore, Hubal et al. (2008) proposed that increased macrophage and satellite cell activity, and/or altered communication between these two cell populations (via MCP-1 or other factors), could significantly contribute to the enhanced recovery observed during the “repeated bout effect” in resistance exercise. Such a proposal is plausible due to the innate roles of macrophages and satellite cells during muscle repair and regeneration. However, due to the extremely damaging nature of the exercise protocol used by Hubal et al. (2008) (i.e. 300 maximal eccentric contractions in untrained adults), such responses will also need to be confirmed during the “repeated bout effect” to more ecologically valid resistance exercise protocols. As MCP-1 is produced by a number of cell types, including monocytes, macrophages, skeletal muscle satellite cells, endothelial cells and fibroblasts (Chazaud et al., 2003; De Rossi et al., 2000; Deshmane et al., 2009; Harmon et al., 2010), the next challenge will be to investigate the localization of MCP-1 protein within tissue on relevant cell types of interest (i.e. satellite cells) during the recovery from high-power, HIRE. Such findings may help to further explain how highly-trained competitive weightlifters can recover from multiple HIRE sessions, involving the same major muscle groups, within the same day.

In the current investigation, CCR2 mRNA levels in PBMCs also exhibited an exercise-induced response (2.5-fold increase) that was attenuated by post-exercise feeding (1.3-fold increase) (refer to Figure 6-3). These findings add to previous evidence that have shown transient changes in genes central to energy homeostasis and metabolic function in PBMCs following short-term changes in feeding, ranging from low calorie diets to periods of
starvation (Bouwens et al., 2007; Caimari et al., 2010; Crujeiras et al., 2008; van Erk et al., 2006). Here, we have shown for the first time that the absence of a post-exercise meal following high-power, HIRE results in a greater expression of CCR2 mRNA in PBMCs 3 h into recovery. Such findings are of interest, as CCR2+ cells are attracted to MCP-1 that is present in areas of damaged tissue, to aid with subsequent repair and regeneration. For example, circulating inflammatory monocytes can infiltrate damaged tissue to aid in phagocytosis (Arnold et al., 2007). Following the removal of cellular debris, these cells are converted to anti-inflammatory macrophages that stimulate and sustain myogenic differentiation and myofiber growth via the secretion of mitogenic factors (Arnold et al., 2007; Sonnet et al., 2006; Tidball et al., 2006). Although such findings suggest that the absence of a post-exercise meal improves the potential for muscle repair and regeneration following high-power, HIRE, it is highly likely that such a practice would negatively affect other key pathways involved with the recovery from, and adaptation to, resistance exercise. For example, a large body of evidence supports feeding soon after resistance exercise to enhance net protein synthesis and subsequent adaptation (Bird et al., 2006; Deldicque et al., 2005; Drummond et al., 2009a; Hawley et al., 2006; 2007; 2010; Hulmi et al., 2009; Koopman et al., 2007; Moore et al., 2009). Thus, a longitudinal study is required to determine if a post-exercise nutritional modulation of MCP-1/CCR2 has functional relevance to the maintenance and/or enhancement of the phenotypic adaptations that are sought by strength and power athletes.

The post-exercise (0 min) increase in serum glucose during the Exercise and Meal trial was within the normal postprandial range of 5.0 – 8.0 mmol/L (American Diabetes Association). However, as anticipated, the serum glucose levels during the Exercise and No Meal trial did not differ from those of the No Exercise and No Meal trial at any stage. Although minor differences in post-exercise glucose levels existed between trials (refer to Figure 6-6), it is unlikely that these differences alone contributed to the differential expression of MCP-1 and CCR2 mRNA due to the mixed macronutrient content of the standardized pre- and post-exercise meals (54.6 % carbohydrate, 19.0% protein, 26.4% fat). In support of this statement, Nieman et al. (2004) demonstrated that carbohydrate ingestion, during a 2 h intensive resistance exercise session, did not alter the modest increases in the mRNA expression of plasma and skeletal muscle inflammatory cytokines (IL-6, IL-10, IL-1ra, IL-8 and TNF-α) when compared with a placebo. Thus, it is likely that the combination of macronutrients
and/or the total energy availability (i.e. calories) post-exercise was the major influencing factor on the blunting of MCP-1/CCR2 mRNA expression.

Despite evidence that a modest degree of muscle damage was sustained during the two exercise trials, no transcriptional response of CXCR4 in PBMCs or SDF-1 in skeletal muscle was found after the high-power, HIRE protocol (refer to Figure 6-3 and 6-4). This is in contrast to the results of Study 1 where high-power, HIRE performed by competitive weightlifters resulted in a robust transcriptional response of CXCR4 in circulating PBMCs, coincident with an increase in indices of skeletal muscle damage. In the current investigation, SDF-1 and CXCR4 mRNA expression levels were determined following singular training sessions in which participants were instructed to refrain from all exercise for at least 48 h prior to each experimental trial. Although the exercise sessions were deemed to be “very hard” based upon the participants’ RPE scores (~17 – 18), it is possible that the modest degree of muscle damage that was sustained (as determined by serum CK levels) was insufficient to elicit an exercise-induced response in CXCR4 and SDF-1. In contrast, during Study 1, competitive weightlifters demonstrated a 3.4-fold change in CXCR4 mRNA from pre- to 3 h post-exercise, but only following a 2 wk overload period. The increased transcriptional response of CXCR4 coincided with a 35% increase in protein carbonyl levels (an early indicator of tissue damage), significant decrements in muscular performance and an increase in negative signs and symptoms (e.g. increased “muscle pains”, “unexplained aches” and “swellings”) associated with physiological stress. Furthermore, it is possible that the expression of CXCR4 and SDF-1 may have increased later than examined here (i.e. >3 h post-exercise). For example, Griffin et al. (2010) demonstrated that the expression of CXCR4 mRNA in primary mouse myoblasts did not occur until 24 h post-injury. In addition, Brzoska and colleagues (2012) demonstrated that expression of SDF-1 mRNA and protein increased during the myoblast differentiation phase which began ~3 days after denervation and crush injury in mouse skeletal muscle.

To the best of our knowledge, this is the first investigation to examine the SDF-1 response in human skeletal muscle following any form of exercise. As SDF-1 is known to positively influence muscle regeneration via the mobilization of CXCR4+ cells from various regions within the body (Brzoska et al., 2012; Kucia et al., 2004; Ratajczak et al., 2003b), further research is required to establish the protein levels and functional roles of the SDF-1/CXCR4 pairing in different tissue and cell types (e.g. skeletal muscle satellite cells) during the
recovery from HIRE. Recently, Brzoska and colleagues (2012) demonstrated that local administration of SDF-1 protein at the site of muscle damage increased the efficiency of muscle regeneration when compared with control tissue (i.e. damaged muscle injected with physiological saline). The improvements in muscle regeneration (via exogenous SDF-1 administration) were characterized by an increased muscle mass, decreased fibrosis, improved tissue architecture and enhanced expression of myogenic markers (Brzoska et al., 2012). Thus, the efficacy of tactics designed to increase SDF-1 levels as a potential intervention to enhance post-exercise recovery is also an area requiring further research.

Finally, although aerobic exercise-induced increases in cortisol have been shown to up-regulate the expression of CCR2 and CXCR4 on PBMCs in a dose-dependent manner (Nagatomi et al., 2002; Okutsu et al., 2002; 2005; 2008), no such association existed between serum cortisol levels and the relative change, or lack thereof, in CCR2 and CXCR4 expression. In light of these results, it is possible that either: 1) the exercise-induced increase in cortisol failed to reach the threshold required to influence CXCR4 and/or CCR2 mRNA expression in PBMCs; and/or 2) the positive influence of the glucocorticoid receptor pathway on CXCR4 and CCR2 expression may be particular to aerobic exercise (Nagatomi et al., 2002; 2002; Okutsu et al., 2005; 2008).

**Technical Considerations**

In the current investigation, we chose a “mixed macronutrient” post-exercise meal to replicate the recommended practices for strength and power athletes (Economos et al., 1993; Rodriguez et al., 2009b). Therefore, it is difficult to determine what influence, or lack thereof, each macronutrient had on the genes examined in the current investigation. Thus, future investigations could examine the influence of consuming, or abstaining from, a post-exercise meal comprised of a single macronutrient or a combination of macronutrients of varying proportions. A second technical consideration is the possibility that the PBMC mRNA results obtained 3 h post-exercise may have been influenced by variations in lymphocyte and monocyte count. However, such an influence is likely to have been minimal as transient changes in total leukocyte count generally return to baseline levels within 2-3 h following resistance exercise (Mayhew et al., 2005; Natale et al., 2003; Nieman et al., 2004; Simonson et al., 2004b; Tvede et al., 1989). Furthermore, Nieman et al. (2004) demonstrated that the ingestion of carbohydrate or a placebo did not alter the pattern of change in lymphocyte
counts following resistance exercise. Thus, the reported difference in PBMC CCR2 mRNA expression between the Exercise and Meal and Exercise and No Meal trials is a genuine transcriptional response to the exercise intervention and the availability of nutrients post-exercise. A third technical consideration is the relatively small sample size used in the current investigation ($N = 7$) due to the lack of available volunteers that met the study requirements. However, the statistical power of this investigation was improved by the use of a within-subject repeated measures experimental design.
6.6 Conclusion

The current investigation shows that post-exercise nutrient availability influences the transcriptional response of genes involved with muscle repair and regeneration pathways. Acute bouts of high-power, HIRE resulted in a robust increase in MCP-1 mRNA in skeletal muscle and the associated receptor, CCR2 in PBMCs (3 h post-exercise). As hypothesized, these exercise-induced transcriptional responses were attenuated by a post-exercise meal. In addition, MCP-1 protein levels in skeletal muscle were increased following the exercise protocol in resistance-trained males, but were unaffected by a post-exercise meal. Since the nutritional-modulation of the MCP-1/CCR2 pairing has relevance to muscle repair and regeneration, further research is warranted in this area.

Despite expectations, no apparent exercise-induced transcriptional response occurred for the SDF-1/CXCR4 pairing during the early recovery phase (i.e. 3 h post-exercise) following high-power, HIRE. However, it is possible that SDF-1/CXCR4 expression increased at a later stage of recovery. Therefore, further research is required to determine the responsiveness of these genes in relevant tissue and cells types (e.g. skeletal muscle satellite cells) at later time points during the recovery from HIRE. As the MCP-1/CCR2 and SDF-1/CXCR4 pairings are important modulators of muscle repair and regeneration, a greater understanding of the chemokine response to HIRE may lead to novel post-exercise recovery interventions. Finally, as nutrient availability pre- and post-exercise serves as a potent modulator of many acute responses and chronic adaptations to resistance exercise, the investigation of nutrient-exercise interactions that have the ability to upregulate or inhibit the pathways involved with training adaptations needs to be pursued.
7.0 GENERAL DISCUSSION
The frequency of HIRE performed by competitive weightlifters is unmatched by other athletes and exceeds the current ACSM recommendations for strength and power training (as detailed in Section 2.3.2 and 2.3.3). Yet, in response to their intensive training structures that involve multiple daily HIRE sessions, and repeated cycles of overload and recovery weeks, weightlifters demonstrate both acute and long-term improvements in the performance of the competitive lifts and other related strength exercises (Crewther et al., 2010; Crewther et al., 2011; Drechsler, 1998; Fry et al., 1994a; Häkkinen et al., 1988b; Pistilli et al., 2008; Stone et al., 1998). As such, the studies in this thesis have helped to explain how highly-trained competitive weightlifters recover rapidly between repeated HIRE sessions within the same day, and with recovery training after short-term periods of overload.

As previous physiological research in competitive weightlifters has examined the acute neuromuscular and/or hormonal responses following single training sessions (Häkkinen et al., 1988a; Kraemer et al., 1992; Liu et al., 2005; McMillan et al., 1993; Passelergue et al., 1995), the results of this thesis have furthered our understanding of how these athletes respond and adapt to repeated and sustained periods of training. Specifically these studies have shown: (1) key systemic responses that are associated with the recovery from and adaptation to variations in prescribed training load in competitive weightlifters (Study 1); (2) skeletal muscle structural and functional responses to “double-day training” between competitive weightlifters and resistance-trained adults (Study 2); and (3) the influence of post-exercise feeding on the responsiveness of inflammatory chemokine receptor pairings that have known links to muscle repair and regeneration pathways following high-power, HIRE (Study 3). A multi-disciplinary approach, incorporating several methodological techniques not previously utilized in the context of high-power, HIRE and/or competitive strength and power athletes, was undertaken to elucidate the systemic, structural and functional responses in each investigation.
7.1 Molecular Responses to High-Power, High-Intensity Resistance Exercise

Evidence is mounting to support the concept that chronic adaptations in skeletal muscle are proposed to arise from the summation of the effects of many single (acute) bouts of exercise which leads to cumulative alterations in gene and protein expression (Pilegaard et al., 2000; Stepto et al., 2009). Such a change in the “genetic set point” (Stepto et al., 2009) and the corresponding function of highly-trained individuals indicates that the expression of key genes involved with long-term adaptation to exercise are chronically altered (Flück, 2006; Stepto et al., 2009). Thus, this thesis examined the molecular responses to HIRE in highly-trained individuals. To obtain valid and meaningful physiological data, a great deal of time and effort was directed towards the development of ecologically valid experiments and the recruitment of appropriately-trained participants.

A major strength of Study 1 was the recruitment of seven International-level competitive weightlifters for the within-subject, repeated measures study design. To the best of our knowledge, this investigation was the first of its kind conducted in highly-trained strength and power athletes during a real-life training period involving prescribed variations in training intensity and volume. The major findings of Study 1 were that 2 wk of overload training (lifting 36,508 ± 11,299 kg/wk) resulted in a robust exercise-induced transcriptional response in genes associated with immunological and inflammatory stress and cell cycle regulation in the PBMCs of these athletes. Furthermore, the intensified training resulted in indices of skeletal muscle damage coincident with decrements in muscular performance and a negative affective response to training. Conversely, restoration of performance occurred after a subsequent week of recovery training (lifting 27,056 ± 8,442 kg/wk) in conjunction with a decrease in basal indices of muscle damage, no exercise-induced response of selected candidate genes in PBMCs, and an improvement in reported mood and stress parameters.

Of particular interest were the robust exercise-induced increases in CXCR4, CCL4 and DDIT4 mRNA expression following the 2 wk overload period. The increased expression of CXCR4 and CCL4 were interpreted as an increase in systemic inflammation arising from exercise-induced tissue damage. Conversely, it was interesting to note that the increase in DDIT4 expression coincided with self-reported negative changes in daily diet and body weight, as previous evidence has demonstrated that DDIT4 is upregulated during periods of energy stress and starvation (Drummond et al., 2008a; Sofer et al., 2005). These results have
relevance to the maintenance and/or enhancement of the phenotypic adaptations sought by strength and power athletes as DDIT4 is a major negative regulator of the mTOR pathway (Deldicque et al., 2005; Drummond et al., 2009b; Ma et al., 2009; Miyazaki et al., 2009). A persistent increase in DDIT4 mRNA expression would have a negative impact on protein synthesis and subsequent muscle repair and growth, thereby reducing the potential for long-term improvements in athletic performance.

By default, the absence of an exercise-induced response of CXCR4, CCL4 and DDIT4 following the recovery week suggested that the overload was essential in the highly-trained competitive weightlifters to induce significant increases in gene expression that lead to functional adaptations. Figure 7-1 depicts how repeated cycles of overload and recovery are used to stimulate improvements in performance via cumulative alterations in gene expression. These findings add to previous evidence demonstrating that the intensity and volume of loading and the number of muscular contractions appear to be the dominant stimuli for muscular adaptation (Flück et al., 2003; 2006). Furthermore, experienced resistance-trained adults require a far greater overload stimulus to induce significant increases in mRNA abundance, when compared with non-habitual resistance trainers (Coffey et al., 2006a; 2006b).
**Figure 7-1:** A summary of proposed performance changes during repeated cycles of overload and recovery training. Improvements in performance (i.e. adaptation) occur due to the summation of effects that arise from many single (acute) bouts of exercise which lead to a cumulative, global alteration in mRNA and protein expression. The large black arrows represent significant increases in mRNA abundance that occur during short-term periods of overload.
As muscle injury is associated with the loss of functional strength (Rathbone et al., 2003; Warren et al., 1999; 2004), the enhancement of muscle regeneration processes following damaging exercise is of great significance to strength and power athletes. Thus, the increased expression of CXCR4 mRNA in the PBMCs of weightlifters following 2 wk of overload was of importance due to the essential functions CXCR4 and its ligand SDF-1 have in muscle repair and regeneration processes (Kucia et al., 2004b; Ratajczak et al., 2006). As little is known about the interaction between inflammatory responses in skeletal muscle and PBMCs following high-power, HIRE, the expression of SDF-1 in muscle following such exercise was investigated in Study 3. The MCP-1/CCR2 chemokine/receptor pairing was also examined in parallel due to MCP-1’s regulation by pro-inflammatory stimuli (Henningsen et al., 2011) and the established role of MCP-1/CCR2 in muscle repair and regeneration (Summan et al., 2003b; Warren et al., 2004; Yahiaoui et al., 2008).

Despite expectations, there were no changes (3 h post-exercise) in the expression of the SDF-1/CXCR4 chemokine/receptor pairing in resistance-trained men following exercise similar to that performed by competitive weightlifters. However, qPCR analyses revealed that the high-power, HIRE resulted in a 6.8-fold increase in MCP-1 mRNA in skeletal muscle and a 2.5-fold increase in the mRNA of the associated receptor, CCR2 in PBMCs (3 h post-exercise). In addition, MCP-1 protein levels in skeletal muscle were increased following both the Exercise and Meal and Exercise and No Meal trials. Such findings are of interest as CCR2+ cells, such as monocytes, are attracted to MCP-1 that is present in areas of damaged tissue, to aid with subsequent repair and regeneration (Warren et al., 2004). As hypothesized, the exercise-induced transcriptional responses of MCP-1 and CCR2 were attenuated by a post-exercise meal. However, post-exercise feeding did not affect the exercise-induced expression of MCP-1 protein in skeletal muscle.

The dual quantification of the molecular responses to high-power, HIRE in the skeletal muscle and blood of resistance-trained adults has furthered our understanding of the interaction of chemokine signalling across these tissue types during the recovery from exercise, similar to that performed by competitive weightlifters. As competitive athletes are often resistant to invasive research procedures (i.e. skeletal muscle biopsy), resistance-trained adults who were competent in the specific resistance exercises required for the investigation were recruited for Study 3.
When viewed in isolation, the feeding-induced attenuation of the mRNA responses of MCP-1 and CCR2 suggest that the absence of a post-exercise meal improves the potential for muscle repair and regeneration following high-power, HIRE. However, in light of the large body of evidence that currently exists in support of feeding directly after resistance exercise (Bird et al., 2006; Deldicque et al., 2005; Drummond et al., 2009a; Hawley et al., 2006; 2007; 2010; Hulmi et al., 2009; Koopman et al., 2007; Moore et al., 2009), such a practice is likely to inhibit other myogenic pathways that are strongly associated with positive adaptation to resistance exercise. For example, as the competitive weightlifters in Study 1 exhibited an increased expression of DDIT4 mRNA (a negative regulator of mTOR signalling) during periods of self-reported negative dietary changes, it is possible that DDIT4 mRNA was also increased during the Exercise and No Meal trial in Study 3. Thus, a longitudinal study is required to determine the functional relevance of an increased expression of MCP-1/CCR2 in the absence of feeding following high-power, HIRE.

### 7.2 Performance and Neuromuscular Responses

In response to the 2 wk overload period in competitive weightlifters, maximal snatch and vertical jump performance were significantly reduced by ~4% and ~7%, respectively. It was anticipated that acute changes in maximal snatch and vertical jump performance would be interrelated as previous evidence has demonstrated a strong kinematic relationship during the propulsive phases of these two movements (Canavan et al., 1996; Carlock et al., 2004; Garhammer et al., 1992; LeFavi et al., 2011). As such, estimated vertical jump peak power (W) was strongly correlated with snatch performance across all time points ($P < 0.001$; $R^2 = 0.9$). These results suggest that changes in maximal vertical jump could be used as a predictor of maximal snatch performance in weightlifters as previously indicated (Carlock et al., 2004). Interestingly, mean clean and jerk performance did not change with overload.

As several studies have suggested that contractile speed may be attenuated more than muscular strength in response to HIRE overload (Fry et al., 2006b; Haff et al., 2008; Häkkinen, 1992), the kinematic differences between the snatch and the C&J may account for the performance decrements, or lack thereof. For example, during the performance of near maximal to maximal
attempts of the snatch, the vertical velocity of the barbell ranges from 1.65 to 3.00 m/sec (Akkus, 2011; Garhammer, 1991; Gourgoulis et al., 2002; Winchester et al., 2009). Conversely, during the performance of near maximal to maximal attempts of the C&J, where ~18% to 20% greater loads are used, the maximum vertical velocity of the barbell may be ~17-47% less than that of the snatch (Garhammer, 1985, 1991; Winchester et al., 2005). In future investigations, it would be worthwhile to compare the changes in competitive weightlifting performance with valid, non-technique dependent measures of neuromuscular performance (i.e. maximal isometric front squat) to confirm whether a greater deficit in the contractile rate of force development (RFD) occurs in relation to peak force during periods of overload.

Following the 1 wk of recovery, maximal snatch and vertical jump performance were restored to personal best/baseline levels (refer to Table 4-3). This restoration of performance indicates that rapid systemic mechanisms (as discussed in Section 7.1) enable weightlifters to positively adapt following a shorter recovery interval (i.e. 1 wk) when compared to other athletes. For example, it may take between 2-5 weeks for the restoration and/or an increase in performance to occur in other athletes (e.g. swimmers, cyclists, American football players) following the cessation of overload periods ranging from 1-3 wk in duration (Fry et al., 1997; Halson et al., 2004; Moore et al., 2007; Stone et al., 1998).

The training of competitive weightlifters is characterized by the frequent use of HIRE over the course of multiple training sessions within the same day. Study 2 established that weightlifters possess a greater ability to generate and sustain peak force (PF) during repeated bouts of HIRE, involving the same major muscle groups, when compared to resistance-trained adults. Despite expectations, the maintenance (or enhancement) of muscular function within and between successive bouts of HIRE was not attributable to acute changes in the architecture of the vastus lateralis (Mahlfeld et al., 2004; Tillin et al., 2009). Thus, other physiological factors such as alterations in motor unit recruitment, firing frequency and/or synchronization are likely to contribute to the ability of competitive weightlifters to perform multiple HIRE sessions within the same day.

As the intention of Study 2 was to make a direct comparison between the acute exercise-induced responses of competitive weightlifters and resistance-trained adults, the exercise stimulus of
choice was a major consideration. Competitive weightlifters routinely perform complex, multi-joint movements (e.g. hang/power/full snatches, hang/power/full cleans, jerks and various pulling and pressing movements) across successive training sessions within the same day. However, the repeated use of similar, highly complex resistance exercises is not necessarily common to resistance-trained adults. Thus, the dynamic front squat was chosen as the exercise stimulus because it is widely used by both cohorts. Furthermore, this line of thought also lead to the development of a non-technique dependent method for measuring muscular function (i.e. isometric front squat test) that has relevance to competitive weightlifters and can be performed by resistance-trained adults.

A technical consideration for Study 2 is the possibility that the participant’s footwear influenced the PF, contractile RFD and CI results between groups. During training and competition, weightlifters wear specialized shoes that have stiff, noncompressible soles and a raised heel. Although these shoes are habitually worn by weightlifters, they are generally unfamiliar to resistance-trained adults who typically perform heavy lifting exercises in running shoes or other types of athletic shoes (Sato et al., 2012). During the development of Study 2, we did consider the influence of footwear on the performance of the dynamic exercise and isometric testing protocol. However, as Barnett et al. (2001) reported no significant differences in vertical force output between cushioned and uncushioned shoes, all participants were instructed to wear their usual training shoes on all occasions to eliminate the need to get accustomed to unfamiliar footwear. Furthermore, as previous evidence indicates that weightlifting shoes do not contribute to the ability to attain a parallel thigh position during squatting movements, when compared to running shoes (Sato et al., 2012), we were confident that all participants would be able to successfully perform the dynamic and isometric parallel front squat with maximal effort.

Collectively, the results of Study 1 and Study 2 established that competitive weightlifters have a unique ability to rapidly recover following short-term periods of overload and between successive HIRE sessions that are performed within the same day.
7.3 Potential Applications

The results presented in this thesis identified, at multiple levels, physiological variables that contribute to the early recovery and adaptive processes following high-power, HIRE in competitive weightlifters and resistance-trained adults. In light of these findings, there are several aspects of this research that have potential applications to the prescription of high-power, HIRE in well-trained populations.

Quantitative and qualitative athlete monitoring and testing is a critical process to ensure that performance gains occur following extended periods of structured training. During Study 1, the increased self-reporting of negative signs and symptoms associated with stress coincided with changes in systemic inflammation and performance decrements. Furthermore, self-reported negative dietary and body weight changes also occurred coincident with the upregulation of a major negative regulator of mTOR signalling; DDIT4. In light of these findings, coaches and strength and conditioning professionals are encouraged to integrate the POMS and DALDA questionnaires into their training and performance monitoring regimes for strength and power athletes. The early detection of increased stress and negative affective states may prevent the onset of a number of adverse symptoms (i.e. performance decrements and/or injury) that are associated with overload training, thereby facilitating optimal sporting performance.

With regard to quantitative assessment of neuromuscular function, PF, contractile RFD and contractile impulse (CI) are routinely determined using isometric contractions (Blazevich et al., 2008; Chiu et al., 2004; Häkkinen et al., 1988a; 1992). The effectiveness of isometric measurements to assess dynamically induced training adaptations can be enhanced by selecting a body position specific to the dynamic performance of interest (Haff et al., 1997; Stone et al., 2003a; Wilson et al., 1996). Therefore, the development of the isometric front squat test (used in Study 2 and Study 3) provides a non-technique dependent, reliable and valid representation of the neuromuscular responses following commonly prescribed lower body dominant, high-power, HIRE (e.g. front squat, power clean and full clean). Furthermore, as maximal muscle activation is enhanced during isometric bilateral versus unilateral contractions (Behm et al., 2003), the isometric front squat test provides a valid measure of the training induced adaptations that arise from bilateral HIRE.
The results of Study 2 demonstrated that resistance-trained adults were able to maintain contractile RFD, but not PF, across two successive HIRE sessions within the same day. These findings have important practical implications for the prescription of HIRE programs that are intended to improve muscular strength and power in resistance-trained adults. For example, resistance-trained adults are likely to benefit more from a single high-intensity strength training session within a given day due to their inability to maintain PF across two successive HIRE sessions. However, due to their ability to maintain and/or improve contractile RFD and CI across successive training sessions, it would be possible for resistance-trained adults to perform a second low-moderate intensity, high-power resistance exercise session within the same day.

Finally, although the results of Study 3 suggest that a post-exercise meal following high-power, HIRE attenuates muscle repair and regeneration processes (via a blunting of the MCP-1/CCR2 chemokine/receptor pairing response), further investigation is required in this area prior to suggesting any potential applications to exercise prescription or post-exercise nutrition.
8.0 FUTURE RESEARCH DIRECTIONS AND CONCLUSIONS
8.1 Future Directions

The series of experiments and studies presented in this thesis documented the mRNA responses of CXCR4, DDIT4, SDF-1 and the mRNA and protein responses of CCL4/MIP-1β and MCP-1 during the early phase of recovery from high-power, HIRE. The next challenge will be to link early exercise-induced gene expression and signalling responses that occur in skeletal muscle and peripheral blood to chronic training adaptations in highly-trained strength and power athletes.

To help inform the direction of such research, it would be advisable to perform microarray analyses on a larger cohort of competitive strength and power athletes during similar overload and recovery training periods. Furthermore, the inclusion of a tapering period, multiple post-exercise sampling time points (e.g. 0 min, 3, 6, 24 h) and comprehensive assessment of neuromuscular function (i.e. isometric PF, contractile RFD and CI) in such investigations will further our understanding of the acute genomic responses that are associated with recovery from high-power, HIRE.

As the absence of a post-exercise meal was shown to have a positive effect on the expression of genes (MCP-1 and CCR2) associated with muscle repair and regeneration following exercise, further investigations need to examine the efficacy of post-exercise interventions that have the potential to upregulate MCP-1/CCR2 expression. Such investigations could examine and compare the effects of consuming varying proportions of macronutrients post-exercise and/or alterations in the timing of delivery. Furthermore, as MCP-1 protein has been shown to be colocalized with macrophages and satellite cells within injured muscle (Hubal et al., 2008), further investigation is required to determine if such a response occurs during the recovery from high-power, HIRE. Such research would add to the work of Hubal et al. (2008), which proposed that increased macrophage and satellite cell activity, and/or altered communication between these two cell populations (via MCP-1 or other factors), could significantly contribute to the enhanced recovery observed during the “repeated bout effect”.

Finally, from the limited available data comparing sex-related differences in the genomic responses to HIRE, it appears that genes play a more prominent role in male than female
strength determination (Beunen et al., 2003; Beunen et al., 2006; Roth et al., 2002). Thus, the proposed directions for future research that are mentioned above also need to factor in the influence of sex-related differences in the acute responses to HIRE.

8.2 Conclusions

This thesis characterizes and begins to explain the remarkable ability of competitive weightlifters to perform and rapidly recover from successive sessions of high-power, HIRE within the same day, and after overload and recovery training. The results of Study 1 demonstrated that a robust exercise-induced response of the selected candidate genes, CXCR4, CCL4 and DDIT4, occurred after 2 wk of overload training. Furthermore, performance decrements observed during the overload period coincided with increases in indices of tissue damage and self-reported adverse signs and symptoms associated with stress in these athletes. Of particular interest were the robust exercise-induced increases in CXCR4 and CCL4 mRNA expression due to the established roles of these chemokines in muscle repair and regeneration. However, a subsequent 1 wk recovery period resulted in no exercise-induced transcriptional response of the candidate genes, a restoration of performance, a decrease in tissue damage and an improvement in reported mood and stress parameters. Thus, the results of Study 1 implicate chemokine signalling in the early recovery response following high-power, HIRE overload training in competitive weightlifters. In addition, these finding suggest that cycles of overload and recovery training are essential to induce gene expression changes that lead to functional adaptations in these athletes.

As hypothesized, competitive weightlifters demonstrated a greater ability to sustain peak force across repeated HIRE bouts of the same muscle group when compared to resistance-trained adults. However, both highly-trained weightlifters and resistance-trained adults sustained or increased their contractile rate of force development and contractile impulse in response to such training. Despite expectations, the ability of weightlifters to maintain (or enhance) muscular function within and between successive bouts of HIRE was not attributable to acute changes in skeletal muscle architecture. Thus, other physiological factors such as alterations in motor unit recruitment, firing frequency and/or synchronization are likely to contribute to the ability of competitive weightlifters to perform multiple HIRE sessions within the same day.
Finally, the results of Study 3 demonstrated that nutrient availability following an acute bout of high-power, HIRE, similar to that performed by competitive weightlifters, has the potential to affect the expression of chemokines associated with muscle repair and regeneration. A robust increase in MCP-1 mRNA in skeletal muscle and the associated receptor, CCR2 in PBMCs occurred in highly-trained adults 3 h post-exercise. However, these exercise-induced transcriptional responses were attenuated by the inclusion of an immediate post-exercise meal. In addition, MCP-1 protein levels in skeletal muscle were increased following the exercise protocol, but were unaffected by a post-exercise meal. Despite expectations, no apparent transcriptional response occurred for the SDF-1/CXCR4 pairing during the early recovery phase (i.e. 3 h post-exercise) following an acute bout of high-power, HIRE. It is possible that the modest degree of exercise-induced muscle damage that was sustained was insufficient to elicit a response in CXCR4 and SDF-1. Since the nutritional-modulation of the MCP-1/CCR2 pairing has relevance to muscle repair and regeneration, further research is required to determine the functional relevance of such chemokine and exercise-nutrient interactions in competitive strength and power athletes.

The assessment of gene expression changes in elite-level athletes is undoubtedly the critical step in furthering our understanding of key factors that contribute to enhanced athletic performance. Independently and collectively, this thesis contributes to the understanding of the complex molecular and neuromuscular responses and adaptations that occur during the recovery from high-power, HIRE in competitive weightlifters and resistance-trained adults.
9.0 APPENDICES
9.1 Appendix A: Glossary of Terms

In this thesis, the following terms are defined as follows;

**Aerobic exercise:** Cyclic and dynamic exercise such as walking, running, swimming or cycling that is performed over a long duration and is aimed at improving health and fitness.

**ANOVA** Analysis of variance

**Concentric contraction:** A type of muscle contraction whereby the muscle shortens while generating force.

**Eccentric contraction:** A type of muscle contraction whereby the muscle lengthens under tension whilst generating force.

**Exercise:** Structured physical activity performed to sustain or improve health and fitness parameters.

**Genomic response:** A detectable change in the mRNA expression of a selected gene in response to a stimulus.

**High-frequency resistance exercise:** Performing structured resistance exercise ≥5 times per week.

**High-intensity resistance exercise:** Resistance exercise performed at ≥80% of one repetition maximum.

**High-power resistance exercise:** Dynamic resistance exercise performed in an “explosive” fashion aimed at improving maximal muscular power.

**Isometric contraction:** A type of muscle contraction whereby force is generated with no change in muscle length.

**Macronutrient:** Relating to dietary carbohydrate, protein or fat.
Moderately-trained: An individual that is currently performing structured exercise (i.e. aerobic and/or resistance exercise) of a low- to moderate-intensity on a semi-regular basis (i.e. 1-2 times per week).

Neuromuscular performance: Relating to the ability to produce voluntary muscular force.

Peripheral blood: Venous blood obtained from an antecubital vein.

Peripheral blood mononuclear cells: Lymphocytes and monocytes.

Post-prandial state: Relating to the period following the consumption of food.

Repeated bout effect: The attenuation of exercise-induced muscle damage following repeated application of the exercise stimulus.

Resistance exercise: Structured weight training exercises performed with either free weights or machine weights.

Resistance-trained: An individual that is currently performing resistance exercise on a regular basis (i.e. ≥2 times per week) and has been doing so for the past 6 months.

Sedentary: An individual that is not physically active on a daily basis.

Transcriptional response: A detectable change in the mRNA expression of a selected gene in response to a stimulus.

Untrained: An individual that has not performed any form of structured exercise but is physically active on a daily basis.

Weightlifter: An individual who trains for and competes in the sport of competitive weightlifting.
| **Weightlifting:** | Solely relating to the sport of competitive weightlifting. |
| **Well-trained:** | An individual that is currently performing structured exercise on a regular basis (i.e. ≥2 times per week) and has been doing so for the past 6 months. |
9.2 Appendix B: Participant Information

STUDY 1:

DEPARTMENT OF SPORT & EXERCISE SCIENCE

REQUEST FOR STUDY PARTICIPANTS

Project Title:
Gene Expression in Blood in Response to Variations in Training Load in Competitive Olympic Weightlifters

ARE YOU:

1) Currently a competitive Olympic weightlifter, aged between 18 and 45 years?

2) Currently training in Olympic weightlifting ≥7 times per week and have ≥2 years training experience?

3) Drug-free and have not failed a random NZ Drug Free Sport Agency drug test at any point?

Purpose of the study:

Genes represent a very small portion of our DNA and encode information about specific cellular structures within our body (i.e. proteins). The aim of the study is to investigate how 2 weeks of increased training load, followed by 1 week of reduced training load (3 week study period), influences the gene expression and metabolic response in blood.

What is involved:

You will be required to follow your normal coach-prescribed training program and two 10ml blood samples will be obtained on 7 occasions over the course of the 3 week study period (resting, pre-exercise and post-exercise samples). You will be required to consume a standardized meal following blood sampling, and perform maximal vertical jump and maximal snatch and clean & jerk attempts at the end of each week. Finally, your height and body weight will be recorded and you will be required to complete 2 brief psychological questionnaires, which will only take a few minutes, on a daily basis.

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 17/6/09 for 3 years, Reference Number 2009/197

Who do I contact if I would like more details?

Adam Storey BSc (Hons), Ph: 027 644 8816; E: adam.storey@auckland.ac.nz

Associate Professor Heather Smith, Ph: (09) 373 7599 extn 84681; E: h.smith@auckland.ac.nz

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PARTICIPANT INFORMATION SHEET

Project Title:
Gene Expression in Blood in Response to Variations in Training Load in Competitive Olympic Weightlifters

Researchers:
Adam Storey BSc (Hons)
Associate Professor Heather Smith (H.O.D. Department of Sport & Exercise Science)

You are invited to participate in the above named study. Genes represent a very small portion of our DNA and encode information about specific cellular structures within our body (e.g. proteins). The aim of the study is to investigate how two weeks of increased resistance exercise (weightlifting) loading, followed by one week of reduced loading, influences the gene expression and metabolic response in blood. Please take the time to think about the information provided below, and feel free to discuss it with your whanau, family or significant other support people, before deciding whether to take part. Taking part is completely voluntary (your choice).

What is the study about?
The study will include 10 experienced competitive Olympic weightlifters (≥2 years training experience) aged between 18 and 45 years. The study will investigate how genes and metabolic measures change over a two week period of increased loading and a one week period of reduced loading during normal training in preparation for a competition. Previous research has shown that long-term resistance training results in a different exercise-induced gene expression response when compared to untrained individuals. This change in “genetic set point” may enable resistance-trained individuals to recovery faster between repeated bouts of resistance exercise. However to date, no study has attempted to examine the physiological mechanism of recovery by changes in gene expression in highly resistance-trained individuals, and in particular Olympic weightlifters.
Am I eligible to participate?
You are eligible to participate in this study if you are an experienced (≥2 years training experience), non-smoking competitive Olympic weight lifter who has met the current OWNZ qualifying standard for National events (C Grade for Senior Men, D Grade for Junior Men & Women and Senior Women), and are aged between 18 and 45 years. Furthermore, you must currently train ≥7 times per week and not use banned performance enhancing substances or methods as per the 2009 WADA World Anti-Doping Code.

You are not eligible to participate in this study if you are;
1) unable to complete the coach-prescribed training programme, 2) have failed a random Drug Free Sport New Zealand (DFSNZ) drug test at any point, or 3) have a known blood borne disease/illness, a known bleeding disorder, past history of bleeding or are currently on medications that prolong bleeding time (e.g. anticoagulant therapy).

If you volunteer for this study, you will be asked to complete a participant checklist to confirm that you are eligible to participate.

What does the study involve?
You will be asked to follow your coach-prescribed training programme which is comprised of repeated three week training blocks (two weeks of increased/heavy loading followed by one week of reduced/light loading). Blood samples will be obtained on seven occasions over the course of a three week training block (“study period”) during a normal competition build up. Blood will be later assayed for changes in gene expression and metabolism.

Two days before the commencement of the three week study period, you will be required to report to the Millennium Institute of Sport & Health (MISH) at 8 AM (as is usual on a Saturday morning) following an overnight fast (refer FIGURE 1). Your height and body weight will be recorded and baseline venous blood samples will be collected in a standard manner. Briefly, the skin overlying a large antecubital vein of the forearm will be cleansed with alcohol and a sterile disposable needle will be inserted resulting in a brief pricking sensation. Two 10mL samples of blood will be drawn from the vein into two vacutainer tubes by a trained investigator or personnel. As with all blood tests, there is a small risk of infection. However, this risk is minimized by following approved blood collection and wound care protocols. Breakfast (cereal, toast and fruit will be available) will be provided to you following the blood sampling procedure and you may begin your normal Saturday morning training after you’ve eaten.

The study period will commence with an increased loading week (Heavy 1). Performance testing will be conducted each Friday during the study period and will include a standard maximum vertical jump test during the morning training and maximum snatch and clean & jerk attempts during the afternoon training. On the last Saturday of the two week increased loading period (Heavy 2), you will be required to report to MISH at 8 AM to have your bodyweight reassessed following an overnight fast. Immediately after the bodyweight reassessment, two pre-exercise venous blood samples (each 10mL) will be obtained in a standard manner. A commercially prepared standardized pre-exercise meal (a Subway sandwich) will then be provided to you. Once you have eaten this meal, you will then be expected to train according to
your programme. Immediately after the completion of this training session, two post-exercise venous blood samples (each 10mL) will be collected from you. Since different genes are expressed at different times in response to exercise, a final two blood samples (each 10mL) will be obtained 3 hours after training. However, to control the influence that food has on gene expression, a commercially prepared standardized post-exercise meal (a Subway sandwich) will be provided to you. Once you have eaten your standardized post-training meal, you will be asked not to eat or drink anything apart from water. You will then be required to stay at MISH until after you have provided the last two venous blood samples for the week. During your stay at MISH, you will be required to keep your physical activity level to a minimum. A selection of DVD’s will be provided for you to watch and you are also able to have a quick shower but must not sauna.

This protocol will be repeated on the Saturday of the one week reduced loading period (Schroeder et al.). Therefore, you will be required to provide two 10mL venous blood samples on seven occasions (total amount 140mL) over the course of three weeks (refer Figure 1). The total amount of blood obtained is 30% of the standard amount given when donating blood to the New Zealand Blood Service during a single visit (1 unit = 470mL).

The blood samples taken during the study will be stored and analyzed at a later date to measure the exercise-induced gene expression response, creatine kinase (a marker of skeletal muscle breakdown), protein carbonyl and F2-isoprostane levels (markers of oxidative damage). Every day for the duration of the baseline, overload and recovery training periods, you will be required to complete the Daily Analysis of Life Demands of Athletes (DALDA) questionnaire. In addition, during the 2 week overload and 1 week recovery period, you will also be required to complete the 65-item Profile of Mood States (POMS) questionnaire. Both questionnaires are simple tests designed to assess how you are responding to the training programme. Once you have completed the study, you will be asked for copies of your usual training session records.
**FIGURE 1:** Graphical summary of the 3 week study period comprised of two weeks of increased training load (Heavy 1 & 2) followed by one week of reduced training load (Schroeder et al.). ☡ 1 = Maximum vertical jump testing (AM); ☡ 2 = Maximum snatch & clean & jerk testing (PM); ▲ 1 = 2 x 10mL Pre-exercise blood samples; ▲ 2 = 2 x 10mL immediate post-exercise blood samples; ▲ 3 = 2 x 10mL 3 hour post-exercise blood samples.

**Accidents and Injury**

In the unlikely event of physical injury as a result of your participation in this study, you may be covered by ACC under the injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. For more details, refer http://www.acc.co.nz. If you have any questions about ACC please feel free to ask the researcher for more information before you agree to take part in this trial or contact ACC directly (04) 918 7700.
Participation

Your participation is entirely voluntary and you may withdraw from participating at any time during the 3 week study period without giving a reason. You have the right to withdraw your data from this study up to 3 days after the final blood sample is obtained at the end of the 3 week study period.

With this type of research, there exists the possibility of ‘incidental findings’ (unexpected findings relating to the participant’s state of health). If something is found that is known to have significant medical implications, you will be put in contact with a medical practitioner. If you do not want to be advised of such findings, then you should not take part in this research.

Any decision to participate or not participate does not affect in any way the relationship you have with the Principal Investigator/Coach (Adam Storey). If you are a staff member or student of the University of Auckland, participation or non-participation will not affect your academic/working relationship with the researchers or your academic grades in any way.

The blood sampling and maximum vertical jump testing will each take approximately 10-15 min and the POMS and DALDA questionnaires will each take approximately 3-5 min. The data obtained from this experiment will be stored on a computer and in hardcopy for a period of up to six years and will be used for presentation and publication in scientific forums. A unique code will be assigned to your data and no material that could personally identify you will be used in any reports. All information and data collected from you will be stored securely, in locked cabinets and on secure computer networks. Only the investigators will have access to this information. However, since the maximum vertical jump, snatch and clean & jerk testing will be conducted in the presence of other athletes this may enable some your results to be known by others. After six years, your data will be deleted from electronic storage and your consent form and all related paperwork will be shredded. Your blood samples will be stored until use or for a period of up to six years in a low temperature freezer in the Exercise Physiology Laboratory at The University of Auckland. Most samples will be completely disposed of during their analyses. However any remaining samples will be destroyed according to standard biological sample disposal methods.

You can request a summary of the study's results, which we can send to you once the project is complete. Potential participants who are Māori are encouraged to consult with their whanau, hapu and/or iwi before giving informed consent. Whanau are invited to participate in discussions regarding the research prior to the consent process, and to attend experimental sessions in support of the participant.

Your consent to participate will be indicated by your signing and dating of the consent form provided to you by the researchers. Signing the consent form indicates that you have freely given your consent to participate, and that there has been no coercion or inducement to participate.

Funding for this project was provided by the University of Auckland, Department of Sport & Exercise.
Who should I contact if I have further questions?

Thank you very much for your time and making this study possible. If you have any further questions please contact either;

**Primary Researcher:** Adam Storey BSc (Hons)  
Department of Sport & Exercise Science,  
Tamaki Campus, Building 734, Room 309  
The University of Auckland  
Private Bag 92019, Auckland Mail Centre, Auckland  
1142, New Zealand.

Ph: 027 664 8816  
Email: adam.storey@auckland.ac.nz

**Head of Department & Main Supervisor:** Associate Professor Heather Smith  
Department of Sport & Exercise Science,  
Tamaki Campus, Building 734, Room 319  
The University of Auckland  
Private Bag 92019, Auckland Mail Centre, Auckland  
1142, New Zealand.

Ph: (09) 373 7599 ext 84681  
Email: h.smith@auckland.ac.nz

**For any queries regarding ethical concerns you may contact:**  
The Chair,  
The University of Auckland Human Participants Ethics Committee  
The University of Auckland, Office of the Vice Chancellor  
Private Bag 92019, Auckland 1142.  
Ph: (09) 373-7599 extn. 83711

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON THE 17th JUNE, 2009 FOR (3) YEARS REFERENCE NUMBER 2009/197
PARTICIPANT ELIGIBILITY CHECKLIST

Project Title:
Gene Expression in Blood in Response to Variations in Training Load in Competitive Olympic Weightlifters

PLEASE READ BEFORE COMPLETING THE CHECKLIST:

The information on this Participant Checklist will enable the researchers to determine if you meet the inclusion criteria for the above named investigation. Please answer all questions truthfully and to the best of your knowledge. No material that could personally identify you will be used in any reports and all information collected from you will be securely stored, in locked cabinets and on secure computer networks. Information on this form will only be known to the investigators of the study and will not be disclosed to any additional parties.

NAME: ___________________________________________ D.O.B: ______
/______ /_______

PLEASE CIRCLE ONE: MALE / FEMALE

ADDRESS: ________________________________________________

_____________________________________________________________________

CONTACT NUMBER: ________________________

EMAIL: ____________________________________________
1) **Are you currently on any form of prescription medication?**

   YES / NO  
   (If YES, please state the name below)

   ► __________________________________________________________
   ► __________________________________________________________
   ► __________________________________________________________
   ► __________________________________________________________

2) **Do you (otherwise) consider yourself healthy?**
   
   No known previous or current heart disease, diabetes, high blood pressure or other major illness? No bleeding disorder, past history of bleeding, not on medications that prolong bleeding time (e.g., anticoagulant therapy)?

   YES / NO  
   (If NO, please elaborate below)

   ► __________________________________________________________
   ► __________________________________________________________
   ► __________________________________________________________
   ► __________________________________________________________

3) **Are you currently free of any illness or infection?**

   YES / NO  
   (If NO, please elaborate below)

   ► __________________________________________________________
   ► __________________________________________________________
   ► __________________________________________________________
To be completed by potential participants of PART B ONLY

4) How many years have you been training specifically for Olympic weightlifting?
______________________ years
(In order to be eligible for this investigation you must be aged between 18 and 45 years and have ≥2 years training experience)

5) Are you currently a competitive Olympic weightlifter?
   If YES, approximately how many times per year do you compete?
   YES / NO ___________________________ times per year
   (Only competitive Olympic weightlifters will be eligible for this investigation)

6) Are you currently able to train upwards of 11 times per week at high relative loads (as per your normal coach-prescribed training programme)?
   YES / NO (If NO, unfortunately you are not eligible for this study)

7) What are your personal best lifts in TRAINING?
   Snatch: ________kg   Clean & Jerk: ________kg
   Date Achieved: __________    Date Achieved: __________
   Front Squat: ________kg   Back Squat: ________kg
   Date Achieved: __________    Date Achieved: ________

8) What are your personal best lifts in COMPETITION?
   Snatch: ________kg   Clean & Jerk: ________kg
   Date Achieved: __________    Date Achieved: __________
   Competition: __________    Competition: ________

9) Briefly describe your competition and representative history (if applicable) (e.g. 3x Under 69kg Senior National Champion (2005 – 2008), NZ Senior representative at the 2007 Oceania Champs - 2nd placing).
   ►__________________________________________________________
   ►__________________________________________________________
   ►__________________________________________________________
10) Do you currently hold any New Zealand (Junior and/or Senior) records? If YES, please elaborate.

<table>
<thead>
<tr>
<th>Age Category (Jnr, Snr)</th>
<th>Weight Class</th>
<th>Record</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Many factors can alter the gene expression within your body. Therefore, please take the time to answer the following questions as accurately and as truthfully as possible. You are not obliged to answer all the questions; however failure to do so will exclude you from the study. All information provided will remain confidential.

11) Are you currently on the Drug Free Sport New Zealand (DFSNZ) list of randomly drug tested athletes?

YES / NO

12) Are you currently or have you ever taken banned performance enhancing substances or used banned performance enhancing methods as per the 2009 WADA World Anti-Doping Code (if needed, please ask the researcher for a copy of the 2009 WADA World Anti-Doping Code)

YES / NO

13) Have you failed a random DFSNZ drug test at any point? If YES, when did this occur?

YES / NO

Date: _____ / _____ / _______
14) Do you currently take any of the following dietary supplements?

<table>
<thead>
<tr>
<th>SUPPLEMENT</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>► Amino Acid Formulas</td>
<td></td>
</tr>
<tr>
<td>► Creatine</td>
<td></td>
</tr>
<tr>
<td>► Endurance Formulas</td>
<td></td>
</tr>
<tr>
<td>► Glutamine</td>
<td></td>
</tr>
<tr>
<td>► Beta-Hydroxy Beta-Methylbutyrate (HMB)</td>
<td></td>
</tr>
<tr>
<td>► Joint Health Formulas (e.g. glucosamine)</td>
<td></td>
</tr>
<tr>
<td>► Meal Replacement Formulas</td>
<td></td>
</tr>
<tr>
<td>► Multi-Vitamins (e.g. Vitamin C or combination formulas)</td>
<td></td>
</tr>
<tr>
<td>► Multi-Minerals (e.g. Zinc or combination formulas)</td>
<td></td>
</tr>
<tr>
<td>► Protein Powder</td>
<td></td>
</tr>
<tr>
<td>► Protein Bars</td>
<td></td>
</tr>
<tr>
<td>► Sports Drinks/Recovery Formulas</td>
<td></td>
</tr>
<tr>
<td>► Stimulants (e.g. caffeine based formulas)</td>
<td></td>
</tr>
<tr>
<td>► Thermogenic/Weight Loss Formulas (i.e. carnitine)</td>
<td></td>
</tr>
<tr>
<td>► Tribulus</td>
<td></td>
</tr>
<tr>
<td>► Other (e.g. nitrates and herbal remedies)</td>
<td></td>
</tr>
</tbody>
</table>

If you answered YES to any of the above, please go to Question 15.
If you answered NO please go to Question 16.

15) Please state the brand name, dosage/serving size, timing of the dosage/serving and the approximate number of dosages/servings per week

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dosage/Serving Size &amp; Additional Information</th>
<th>Timing of Dosage/Serving</th>
<th>Number of Dosages/Servings Per Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. Musashi WPC</td>
<td>30g serve with water and 2 tablespoons of powder glucose</td>
<td>1 x serve 20min before training, 1x serve immediately after training</td>
<td>12</td>
</tr>
<tr>
<td>Protein Powder</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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16) Do you currently take any of the following medications?

MEDICATION YES

► Anti-Inflammatories (e.g. Volatren)
► Cardiac Medication
► Cholesterol Medication
► Insulin
► Oral Contraceptives
► Pain Killers (e.g. Nurofen)
► Sedatives (sleeping pills)
► Ventolin Inhalers
► Other

If you answered YES to any of the above, please go to Question 17.
If you answered NO please go to Question 18.

17) Please state the brand name, typical dosage, typical timing of the dosage and the approximate number of dosages per week.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Typical Dosage</th>
<th>Typical Timing of Dosage</th>
<th>Number of Dosages Per Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. Panadeine</td>
<td>2 x capsules</td>
<td>2 x capsule 20min before training if I have sore knees &amp; 2 x capsules in the evening</td>
<td>3</td>
</tr>
</tbody>
</table>
18) Do you have any food allergies that you are aware of? If YES, please specify below.

► ___________________________________________________________________________

► ___________________________________________________________________________

► ___________________________________________________________________________

Thank you for your time

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON THE 17th JUNE, 2009 FOR (3) YEARS REFERENCE NUMBER 2009/197
CONSENT FORM
THIS FORM WILL BE HELD FOR A PERIOD OF 6 YEARS

Project Title:
Gene Expression in Blood in Response to Variations in Training Load in Competitive Olympic Weightlifters

Researchers:
Adam Storey BSc (Hons)
Associate Professor Heather Smith (H.O.D. Department of Sport & Exercise Science)

I have read the Participant Information Sheet (PIS), have understood the nature of the research and why I have been selected. I have had the opportunity to ask questions and I have had them answered to my satisfaction. I understand that my participation in this study is voluntary and that I have not been coerced into participation.

I agree to;

1. Continue to follow my normal coach-prescribed competition training programme in which I will be required to train upwards of 11 times per week during a 3 week study period (2 weeks of increased training load followed by 1 week of reduced training load).

2. Report to the Millennium Institute of Sport & Health (MISH) at 8am on 3 occasions (Saturday mornings) following an overnight fast to have my height and body weight recorded and to provide resting venous blood samples (2 x 10ml).

3. Consume a standardized meal following the resting blood samples and train according to my normal Saturday morning programme.

4. Provide immediate post-exercise blood samples (2 x 10mL) on the last Saturday of the 2 week increased loading period and on the Saturday of the 1 week reduced loading period.
5. Consume a commercially prepared standardized meal immediately following the post-exercise blood samples (2 x 10mL) as obtained on the 2 occasions mentioned above.

6. Stay at MISH and to keep my physical activity levels to a minimum until after I have provided my final 2 blood samples (2 x 10mL) at 3 hours post-exercise. I will not sauna during this time.

7. Perform a maximum vertical jump test at each Friday morning training session during the three week study period.

8. Perform maximum snatch and & clean & jerk attempts at the Friday afternoon training sessions during the three week study period.

9. Complete the Profile of Mood States (POMS) and Daily Analysis of Life Demands of Athletes (DALDA) questionnaires on a daily basis during the three week study period.

I understand that;

- My participation in this study is entirely voluntary and that I have not been coerced into participation.
- I am free to withdraw participation at any time without providing a reason.
- Minor discomfort (as described in the PIS) is associated with blood sampling.
- All maximal physical testing (vertical jump, snatch & clean & jerk) will be done in the presence of other study participants and athletes and may result in minor discomfort.
- Once I have eaten the standardized meals that will be given to me free of charge by the researchers, I am not to eat or drink anything (apart from water) until after the subsequent blood sampling procedures.
- I am free to withdraw any data traceable to me up to 3 days after the final blood sample is obtained from me at the end of the 3 week study period.
- Any decision to participate or not participate does not affect in any way the relationship I have with the Principal Investigator/Coach (Adam Storey).
- If I am a staff member or student of the University of Auckland, participation or non-participation will not affect my academic/working relationship with the researchers or my academic grades in any way.
• My identity will be kept strictly confidential, and no personal identification of me or my data will be made in any subsequent publication of the research findings.

• Data will be kept for 6 years, after which they will be destroyed.

• I am able to request a summary of the outcome of the full research project from the experimenters upon completion of the study.

• I am encouraged to consult with my whanau/family, hapu or iwi regarding participation in this project. Whanau are invited to participate in discussions regarding the research prior to the consent process.

Please circle the appropriate;

I wish / do not wish to receive a summary of results of the full research project of which this is a part of.

I ________________________________ (Full name) have read and consent to all the points outlined above for the investigation. I have also read and consent to all that is outlined in the Participant Information Sheet (PIS).

Signature ________________________________ Date _________________

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON THE 17TH JUNE, 2009 FOR (3) YEARS REFERENCE NUMBER 2009/197
PARTICIPANT REMINDER LIST

Training:
1) Complete all prescribed training.

2) Refrain from using any performance enhancing substances or anti-inflammatory medications.

Questionnaires:
1) Complete the POMS questionnaire at the same time each morning prior to training and on rest days.

2) Complete the DALDA questionnaire at the same time each afternoon prior to training and on rest days.

Performance Testing:
1) Maximum vertical jump testing each Friday morning before training.

2) Maximum snatch and C&J testing each Friday afternoon.

Blood Sampling
1) Report to MISH at 8am on the following Saturday mornings in a RESTED and FASTED state;
   • Saturday 6th of March
   • Saturday 20th of March
   • Saturday 27th of March
STUDY 2:

REQUEST FOR STUDY PARTICIPANTS

Project Title:
Post Activation Potentiation in Advanced Resistance Trained Participants

ARE YOU:
1) Currently EITHER a competitive Olympic weightlifter or a person who performs regular weight training?
2) Aged between 18 and 45 years and free from injury?
3) Drug-free and have not failed a random NZ Drug Free Sport Agency drug test at any point?

Purpose of the study:
A number of competitive athletes train multiple times per day however, at present there is a lack of information relating to what the appropriate training frequency, volume and intensity is for advanced, resistance trained populations. Post-activation potentiation (PAP) is a physiological phenomenon whereby muscular performance is improved in response to a prior “conditioning contraction”. The significance of PAP to functional performance has not been well established and more importantly, the time-course of the potentiation effects are unknown. This is a critical consideration for athletes who perform two high-intensity training sessions on the same day.

What is involved:
The purpose of this study is to measure the change in muscular force and muscle fibre angle pre and post two bouts of high-intensity resistance exercise performed on the same day. The performance of resistance trained individuals will be compared against competitive Olympic weightlifters with the aim of determining whether or not potentiating effects can be seen during the performance of a second bout of high-intensity resistance exercise on the same day.

Who do I contact if I would like more details?
- Samantha Wong, Ph: 373 7599 ext 86990 ; E: samw66@hotmail.com
- Adam Storey BSc (Hons), Ph: 373 7599 ext 86990 ; E: adam.storey@auckland.ac.nz

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON THE 9TH DECEMBER, 2009
FOR (3) YEARS REFERENCE NUMBER 2009/09
PARTICIPANT INFORMATION SHEET

Post-activation potentiation in advanced resistance trained participants

You are invited to participate in the above named study which is a research based investigation conducted by Mr Adam Storey and Miss Samantha Wong, supervised by Dr. Paul Marshall and Associate Professor Heather Smith.

Background Research and Reason for Study:

Resistance exercise training programmes are widely prescribed to active individuals, competitive athletes and also to those within rehabilitation settings. However, at present there is a lack of information relating to what the appropriate training frequency, volume and intensity is for advanced, resistance trained populations. For example, the American College of Sports Medicine currently recommends a resistance training frequency of 4-5 days per week for advanced training. However, elite-level competitive athletes such as Olympic weightlifters have been reported to train their major muscle groups 2-3 times per day over the course of 6 sessions per week.

Post-activation potentiation (PAP) is a physiological phenomenon whereby muscular performance is improved in response to a prior “conditioning contraction”. For example, complex training is a strategy that applies the principles of PAP and involves the execution of a heavy resistance exercise immediately prior to the performance of an explosive type movement (i.e. heavy back squats paired with plyometric jumps). The significance of PAP to functional performance has not been well established and more importantly, the time-course of the potentiation effects are unknown. This is a critical consideration for athletes who perform two high-intensity training sessions on the same day. Recent studies have also shown that the training status of an individual can affect the manifestation of muscular potentiation or fatigue following resistance exercise.

Ultrasound imagery has shown rapid changes in the alignment angle (pennation) of skeletal muscle fibres in response to resistance exercise. However, it is not clear whether or not these changes in pennation angle are associated with PAP. Therefore, the purpose of this study is to measure the change in muscular force and muscle pennation angle pre and post two bouts of
high-intensity resistance exercise performed on the same day. The performance of resistance trained participants will be compared against competitive Olympic weightlifters with the aim of determining whether or not potentiating effects can be seen during the performance of a second bout of high-intensity resistance exercise on the same day.

Am I eligible to participate?

You are eligible to participate if you aged between 18 and 45 years, are free from any upper or lower body injury which may interfere with performance, free from any metabolic or neuromuscular disease, and are not using any performance enhancing supplements or banned methods (as per the 2010 World Anti-Doping Association’s Prohibited List). We will ask you whether you are currently taking any form of steroids or illegal substances, and if you are using you will be excluded from participation in this study. Potential participants must either be; 1) currently competitive in Olympic weightlifting or 2) have been performing resistance exercise at least one day per week for the last year.

Participation in this study is completely voluntary and participants have the right to withdraw from the study at any time without reason. Participants who wish to withdraw will remain anonymous.

Study Protocol:

If you are selected for the study will be required to attend two testing sessions, separated by 4 to 6 hours where you are able to leave the facility and pursue normal activities (no exercise). Each testing session will include quantitative measures of muscular strength, muscular power, and muscle architecture. Each testing session will also include performance of a free-weight barbell front squat exercise and the total duration of each testing session, including the exercise component, will be approximately one and a half hours. Therefore the total amount of participation during the testing day will be approximately three hours (inclusive of testing and exercise performance).

During the barbell front squat exercise component of each testing session, you may be observed by members of the public who are using the gym at the same time.

Testing Procedures:

You will be required to have an acoustic, water soluble gel applied to the skin above the muscle on the outside of your right thigh prior to ultrasound imaging. This is a non-invasive method of imaging your leg musculature. The applied gel can easily be cleaned off once imaging is completed.

The strength of your right leg will be tested using a fixed position on our BIODEX testing machine. This will involve your body being firmly strapped into the testing chair, and your right
leg set in the testing position. Adequate familiarisation will be provided prior to maximal efforts being performed.

The exercise component of each session will involve performing a free-weight barbell front squat exercise using a percentage of your maximal strength level. Standard maximal strength testing guidelines as prescribed by the American College of Sports Medicine will be adhered to when determining each participant’s maximal strength level. The barbell front squat is a commonly used exercise in the training environment. Safety instructions will be provided prior to performance of the exercise session. This exercise session will be directly supervised by one of the research investigators.

**Study Benefits and Risks:**

You will receive information regarding your maximal muscular strength, maximal power output, and any potential changes in your skeletal muscle architecture (i.e. changes in muscle pennation).

You will be asked to perform two identical high-intensity resistance exercise protocols (unilateral leg press) on the same day which will have the potential to cause fatigue during and at completion of the exercise session. There is a possibility that you may experience transient muscle soreness 12-24 hours after the testing sessions, which may ultimately last 24-48 hours.

If instructions and guidelines for correct exercise technique are not followed, there is a possibility that you may physically injure yourself. Physical injury owing to an accident of this nature may be covered under ACC. You should advise the researchers immediately if you are injured during the testing period so we can evaluate your ability to continue the study. You may increase your risk of harm if you fail to do so.

In the unlikely event of a physical injury as a result of participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention, Rehabilitation and Compensation Act. If their claim is accepted by ACC, you still might not get compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you are provided with coverage by ACC, this will generally affect your right to sue the investigators. You are also advised to check whether participation in this study would affect any indemnity cover you have or are considering, such as medical insurance, life insurance and superannuation.

For further information regarding ACC coverage, the nearest ACC office can be contacted on (freephone 0800 735 566, or online at their website [www.acc.co.nz/claimscare/making-a-claim/medicalmisadventure/index.html](http://www.acc.co.nz/claimscare/making-a-claim/medicalmisadventure/index.html)).

We ask you to provide honest and accurate information throughout the study period.
Data Storage, Withdrawal and Confidentiality:

The electronic data will be saved and password-locked on personal computer files of Adam Storey and Samantha Wong and on computer files at the Department of Sport and Exercise Science. Access to files will be restricted to the aforementioned individuals and supervisors (Dr. Paul Marshall and Associate Professor Heather Smith). Your identity will be coded to ensure computer files do not identify any persons.

All the recorded data will be stored in a locked file cabinet in the Department of Sport and Exercise Science at The University of Auckland for a period of six years. After completion of the study and data analysis, all data will be destroyed.

If desired, you can inform researchers whether you would like access to your data after the training intervention.

You have the right to withdraw from the study at any time, without reason. However, you are unable to withdraw your data any later than 1-month after the testing session as results will have been used for analysis. Any decision to participate or not participate does not affect in any way the relationship you have with the Investigators. If you are a staff member or student of the University of Auckland, participation or non-participation will not affect your academic/working relationship with the researchers or your academic grades in any way.

The data will be used for publications, presentations, and investigations in the future. It will not be possible to identify you or your data in any publication of this work.

Who should I contact if I have further questions?

Thank you very much for your time and making this study possible. If you have any further questions please contact either;

Researcher:  Adam Storey  BSc (Hons), PhD Student  Department of Sport & Exercise Science,  Tamaki Campus, Building 734, Room 309  The University of Auckland  Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand.

Ph: 021 2124200  Email: adam.storey@auckland.ac.nz
Researcher:  
Samantha Wong  
Department of Sport & Exercise Science,  
Tamaki Campus, Building 734  
The University of Auckland  
Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand.  

Email: samw66@hotmail.com

Supervisor:  
Dr. Paul Marshall  
Department of Sport & Exercise Science,  
Tamaki Campus, Building 734, Room 319  
The University of Auckland  
Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand.  

Ph: (09) 373 7599 ext 82378  
Email: p.marshall@auckland.ac.nz

Supervisor/Head of Department:  
Associate Professor Heather Smith  
Department of Sport & Exercise Science,  
Tamaki Campus, Building 734, Room 319  
The University of Auckland  
Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand.  

Ph: (09) 373 7599 ext 84681  
Email: h.smith@auckland.ac.nz

For any queries regarding ethical concerns you may contact:  
The Chair,  
The University of Auckland Human Participants Ethics Committee  
The University of Auckland, Office of the Vice Chancellor  
Private Bag 92019, Auckland 1142.  
Ph: (09) 373-7599 extn. 83711

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON THE 9TH DECEMBER, 2009 FOR (3) YEARS REFERENCE NUMBER 2009/09
PARTICIPANT QUESTIONNAIRE

This Participant Questionnaire will be obtained for a maximum of six years. At the end of this period this document will be destroyed.

Post-activation potentiation in advanced resistance trained participants

A research based investigation conducted by Adam Storey and Samantha Wong, supervised by Dr. Paul Marshall and Associate Professor Heather Smith

1. Full Name........................................................................................................................................

2. Male/Female......................................................................................................................................

3. Date of Birth.....................................................................................................................................

4. Contact Number/s..............................................................................................................................

5. Contact Address..................................................................................................................................

6. Are you currently taking any medications? If so, then list below the name and dosage.
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7. Are you currently taking any supplements in your diet? (Including creatine, protein, caffeine and/or any form of steroids).
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8. Do you have an injury history? What was damaged and when did it happen?
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9. Do you currently have any injuries or functional abnormalities that may interfere with your performance? If so, what are they and when did they occur?
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10. Are there any particular movements or exercises that aggravate pain? If so, what are they?
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11. Have you been exercise training for at least once per week for the last year? If yes, what sort of programmes have you been involved in e.g. circuit, resistance, cardiovascular, sport specific etc.?
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........................................................................................................................................
........................................................................................................................................
12. How often do you complete resistance based exercise?

13. Do you have any other information that you think is relevant to your participation in this study? If so, explain below.

Thank You

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON THE 9TH DECEMBER, 2009 FOR (3) YEARS REFERENCE NUMBER 2009/09
PARTICIPANT CONSENT FORM

This Participant Consent Form will be held for a period of six years.

Post-activation potentiation in advanced resistance trained participants

This study is a research based investigation conducted by Miss Samantha Wong and Mr Adam Storey, supervised by Dr. Paul Marshall and Associate Professor Heather Smith.

I have read the Participant Information Sheet and understand the nature of the research and why I been selected. I have had the opportunity to ask questions and have had them answered to my satisfaction. I understand that my participation in this study is voluntary and I have not been coerced into participation.

I understand that:

- I will be required to perform two identical high-intensity resistance training protocols on the same day at the University of Auckland.
- I am able to withdraw from participation at any time without giving a reason.
- I have the right to withdraw my data up until one month after completion of the testing protocols.
- I may be observed by members of the public who are using the gym at the same time when some testing procedures are taking place.
- The collection of some data requires application of a water soluble transmission gel to the outside of my right thigh.
- My results in hard copy and softcopy will be kept for a minimum period of six years at the Department of Sport and Exercise Science, The University of Auckland.
• My data may be used for publications or further investigations in the future and my name will not be used in the publication of the results.
• **Yes / No** (circle one) I would like to have access to my data at completion of the training intervention.
• Expense funding for consumables is from the Department of Sport and Exercise Science.
• The form of exercise I will be prescribed has the potential to cause physical harm if not carried out correctly. For this reason I agree to carry out the required exercise with care and I will inform the researchers if any injuries arise during the participation of this study. Failing to do so may put myself at increased risk of harm.
• I consent to having provided the researchers with honest and accurate information and will continue to throughout the study.

I .................................................................................... (Full name) consent to all of the above and what I have read in the Participant Information Sheet.

.............................................................................. (Signature)  .............................................................................. (Date)

Thank You

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON THE 9TH DECEMBER, 2009 FOR (3) YEARS REFERENCE NUMBER 2009/09
DEPARTMENT OF SPORT & EXERCISE SCIENCE

REQUEST FOR STUDY PARTICIPANTS

Project Title:
Molecular responses to high-intensity, dynamic resistance (‘power’) exercise and feeding during recovery in resistance trained men

ARE YOU

1) Male, healthy and aged between 18 and 35 years?

2) Currently performing high intensity resistance or ‘power’ exercise at least twice per week for the past 6 months?

3) Drug-free and not currently taking any nutritional supplements?

Purpose of the study:

Little is known regarding the molecular responses to high intensity, high-velocity resistance exercise practiced by athletes in many sports and aimed at improving muscle force at high velocity, or muscle power. Much of the research has used the fasted state as the nutritional environment to study the molecular responses after resistance exercise. However, in practice, few people perform resistance or power exercise without a meal beforehand (fed). The purpose of this study will be to determine the molecular responses in muscle and blood to power training exercise in the fed state, and the effects of a post-exercise meal on those responses, in resistance trained men.

What is involved:

You will be required to answer some questions about your health and training to confirm that you are eligible to participate. Those who meet the eligibility criteria will be asked to complete an exercise familiarisation session and three experimental trials over a 4-8 week period. During each of the three trials, you will provide four 20ml blood samples and one muscle sample. The trials involve eating a standardized breakfast followed by either a 90 min power exercise session or rest only, and then with a meal or no meal for 3 hours afterwards. It will take approximately 24 hours of your time in total.

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 18/08/2010 for (3) years, Reference Number 2010/314

Who do I contact if I would like more details?

| Stefan Weite | E: s.wente@aubk.ac.nz |
| Stefan Weite | Ph: 021 022 0635 |
| Stefan Weite | E: s.wente@aubk.ac.nz |
| Stefan Weite | Ph: 021 022 0635 |
| Stefan Weite | E: s.wente@aubk.ac.nz |
| Stefan Weite | Ph: 021 022 0635 |
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| Stefan Weite | Ph: 021 022 0635 |
| Stefan Weite | E: s.wente@aubk.ac.nz |

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PARTICIPANT INFORMATION SHEET – Volunteers

Project title: **Molecular responses to high-intensity dynamic resistance ('power') exercise and feeding during recovery in resistance trained men**

Name(s) of Researcher(s): Associate Professor Heather Smith (Supervisor), Stefan Wette (Researcher), Adam Storey (Researcher)

Healthy 18-35 year old males are invited to participate in a research project being conducted by the Department of Sport and Exercise Science, University of Auckland. Please read this sheet carefully and be confident that you understand its contents before deciding whether or not to participate. If you have any questions about the project, please ask one of the investigators.

**Who is involved in this research project? Why is it being conducted?**

Assoc Prof Heather Smith is the principal researcher responsible for overseeing the research and supervision of graduate students Stefan Wette and Adam Storey. Stefan Wette is an MSc student in the Department of Sport and Exercise Science. Mr Wette is responsible for the scheduling of study participants and organization and conduct of the data collection sessions. Mr Storey and other trained personnel will assist in the data collection sessions. This project is being conducted to enhance our knowledge of the changes occurring in muscle and blood in response to high intensity 'power' resistance exercise and the effects of eating a meal after the exercise on those responses.

**What is the project about? What are the questions being addressed?**

The complex process of exercise-induced adaptation involves rapid and short-term changes (within minutes to hours of exercise) that lead to the making of more new protein in muscle. The functional outcomes (e.g., increases in muscle strength, power or endurance) of these changes are determined by the nature of the training stimulus.

Little is known regarding the exercise-induced changes to high intensity, dynamic exercise aimed at improving muscle force at high velocity, or muscle 'power' and practiced by athletes in sports such as Olympic weightlifting, the sprint and jump events in athletics, rugby, or basketball. We are addressing two questions: 1) what are the exercise-induced changes in the levels of metabolic, mechanical and muscle remodelling-related genes in muscle after performing high-intensity dynamic resistance ('power') exercise, and 2) whether, and in what ways, eating a meal directly after the exercise might affect the early changes in the levels of these genes. Information concerning these changes in muscle and blood may explain functional changes in metabolism and muscle structure.
Exercise activates molecular pathways within the muscle that are important to the control of muscle remodelling and metabolism. Greater knowledge of these exercise-induced adaptive pathways and post-exercise feeding is important for our understanding of how exercise alters muscle function and has applications to exercise training practices.

The purpose of this study is to determine the systemic (blood) and local (muscle) changes with ‘power’ resistance exercise, and the effects of a post-exercise meal on those changes, in resistance exercise-trained men.

**If I agree to participate, what will I be required to do?**

Volunteers will be required to make a total of four visits to the Sport and Exercise Science Department at the University of Auckland, Tamaki Campus. It will take a total of approximately 24 hours of your time comprising one familiarization session and three experimental trial days to complete the study. The familiarization session will take approximately 2-3 hours, and each experimental trial will take approximately 7 hours (from morning to late afternoon each day) to complete. For each experimental trial, your travel costs will be compensated by petrol/grocery vouchers. You will be required to provide written consent prior to your participation in the project.

**Familiarization Session**

You will be asked to complete the Pre-Participation Questionnaire, asked other questions regarding your resistance training experience and whether you have any food allergies. You will be required to complete the following measurements during this session:

1. **Maximal strength test: Back Squat 1 repetition maximum (1-RM)**
   The aim is to find your 1-RM in 3-5 maximal efforts. After warm-up sets using sub-maximal loads, you will be required to attempt to lift estimated maximal weights until an attempt has failed. Your 1-RM is determined as the highest successful lift.

2. **Power test: You will perform three vertical jump practice trials for warm-up and then three test jumps. Your maximal jump height achieved will be recorded.**

3. **Body Composition: Your height, weight, skinfold thicknesses, and limb and trunk circumferences will be measured using standard procedures.**

**Diet/Exercise Control before and during the experimental trials**

Before an experimental trial (described subsequently), you will be required to refrain from all vigorous physical activity, including resistance exercise, for a minimum of 48 hours. You will be provided with a standardised pre-exercise breakfast containing a mixture of carbohydrate, protein and fat upon arrival at the laboratory, following an overnight fast (>8 hours). You will be required to abstain from caffeine and alcohol on the night before and morning of the trial as this could affect test results. You will be required to complete a food diary for two days before the first experimental trial and will be asked to repeat those meals for the two days before each of the following 2 trials. The experimental trials will be separated by a minimum of 6 days. Other than the 2 days just prior to each experimental trial, between trials you are allowed to perform your habitual training.
Experimental Protocol

The familiarization sessions and the exercise during experimental trials will be performed at the Training Centre located on the University of Auckland Tamaki Campus. The exercise sessions will be supervised and involve series’ of whole-body power exercises for a total of 90 minutes. The remainder of the experimental trials will be completed in the Exercise Physiology Laboratory in the Dept. of Sport and Exercise Science, also at Tamaki Campus. You will be asked to complete one no-exercise trial, and two exercise trials (one with, and one without food after the exercise) in a randomised order. During the ‘no exercise’ trials, you will remain in the laboratory and be allowed to use a computer and/or watch DVDs (a selection will be provided).

For each trial you will be required to eat a standardized meal provided to you before the exercise (or ‘no exercise’) session and immediately following the exercise in one of the exercise trials so that the amount and type of food ingested is the same for all trials and for all participants.

As a participant you will be exposed to some risks arising from invasive sampling techniques (Figure 1). These are explained in full detail below, along with the appropriate measures taken to provide you with the safest possible testing environment. All invasive procedures (muscle biopsy and blood sampling) will be conducted using sterile equipment and supplies.
**Muscle Biopsies**

A skeletal muscle (vastus lateralis) sample (biopsy) will be obtained from the thigh of one leg three hours after the exercise/no exercise within each trial. Therefore, you will have a total of three biopsies (1 or 2 from each leg) over the course of the study. A medical doctor will perform the biopsies. The procedure is done under sterile conditions.

**In detail:** The muscle biopsy will involve the administering of a local anaesthetic to the outside part of the upper thigh. This will be followed by a small incision made through the skin. A 5 millimetre biopsy needle will then be inserted through the incision and into the muscle to remove a small piece of muscle tissue (~100 mg, size of ½ a kernel of corn).
incision will be closed with steri-strips (like a band aid). After the muscle biopsy appropriate recovery processes will be performed including, rest, ice, compression and elevation of the leg.

The muscle biopsy process may result in slight discomfort (a mild "cork"), which may last for the 1-3 days. This may be accompanied by local temporary bruising (although rare), along with the very small risk of superficial nerve damage in the skin (caused by the incision), which if present, may cause a temporary (≈1-10 days) loss of sensation to the area. However, this is also extremely rare. You should not perform vigorous exercise for the next 24-48 hours after a biopsy as this may delay the healing process. We will provide appropriate recovery methods (see below) to aid the healing process. However, you may resume light exercise approximately 24 hours after the procedure. If you get a haematoma due to the biopsy procedure the doctor will apply pressure to the sample area to reduce the bleeding and follow the RICE procedures of rest, ice, compression and elevation. There is the extremely remote risk of a small decrease in muscle size at the site of the muscle biopsy. In the event that the intramuscular bleeding is not reduced using RICE procedures, we will refer you to a medical practitioner. To allow the incision to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 4 days. Daily showers are acceptable, but baths, swimming, saunas etc. should be avoided for at least 4 days following a biopsy. You will be given the contact number of a medical doctor to call if you have any concerns about the biopsy or in the circumstance you feel unwell at any stage during the recovery period.

The muscle sample will be stored in a low temperature freezer in the laboratory in Sport and Exercise Science until it is processed for analysis. The processed muscle will be analysed for mRNA and protein expression levels of metabolic and muscle remodelling-related genes.

Blood Samples

A total of 24 ml (at rest and 3h after exercise) and 8ml (before exercise or no-exercise and after exercise or no-exercise) of venous blood will be collected by trained personnel at each of the four time points during each of the three experimental trials by standard methods. The total volume of blood to be taken during the experimental trial, approximately 64 ml per trial day is less than one fifth of the amount that is given during a standard Red Cross donation. On the exercise trial days, blood samples will be obtained using standard venepuncture techniques. On the no-exercise trial day, blood samples will be obtained using standard cannulation techniques.

Blood will be analysed for substances such as glucose, lactate, insulin, amino acids, fat, cytokines, markers of oxidative stress, enzymes, mRNA and protein levels.

Physical Activity

For the most reliable and valid test results it is important for you to be physically rested. You will be required to perform no intense exercise for 48 hours before the familiarization session and each of the three experimental trials.

What are the risks or disadvantages associated with participation?

Muscle Biopsy: You will experience some discomfort for 24-48 hours after the muscle biopsy, however, this should not restrict movement. In some rare cases mild bruising has occurred but these symptoms disappear within 4-5 days. Although the possibility of infection, scarring and significant bruising is quite small, if by chance it does eventuate,
inform us immediately. Individuals who are pre-disposed to scarring may not wish to participate.

Possible side effects you may experience from the muscle biopsy include bleeding, collection of blood under the skin, bruising or infection. By applying proper pressure over the site, bleeding and bruising are usually avoided. Strict sterile precautions will be taken to avoid infection, which is therefore extremely rare. Pain is unlikely since a local anaesthetic is used. However, you may experience a deep pressure feeling at the site of the biopsy. You will have temporary discomfort for 1 to 3 days following the procedure at the site where the incision was made. In very rare instances, discomfort or numbness around the biopsy site may occur for up to one year or longer. To minimise risks of bleeding, vigorous exercise should be avoided for the first 3 days after each muscle biopsy. A small, permanent scar is often visible following muscle biopsy and if skin nerve injury happens, it can be permanent.

**Blood sample:** Venous blood samples will be collected according to standard procedure. Briefly, the skin overlying a large antecubital vein of the forearm will be cleansed with alcohol and a sterile disposable needle will be inserted causing a brief pricking sensation. A total of 64ml per trial day of venous blood will be drawn from the vein into vacutainer tubes. As with all blood tests, there is a small risk of infection. However, this risk is minimized by following standard blood collection and wound care protocols. Trained personnel will perform the blood sampling according to standard safety procedures.

**Exercise:** While all strenuous physical exertion involves some possible risk of injury or complication the exercise sessions will be prescribed and supervised by the researchers, at least one of whom will have First Aid training, and include a thorough warm-up so as not to present any greater risk than that of a typical, intense exercise training session. You may experience some local muscle soreness after the exercise; this soreness normally resolves within 2-3 days. There is also a small risk of musculoskeletal injury with the exercise. The University of Auckland training facility where the exercise will be performed has established emergency procedures and trained staff available to assist if needed.

**What are the benefits associated with participation?**

Participants will have the opportunity to receive information about their maximum muscular strength, power and body composition. In discussions with the investigators during the course of the study, participants will have opportunity to learn about the known and possible responses to power exercise and nutrition that may inform some of their own training practices. You will also be given a $20 petrol/food voucher on each of the experimental trial days to cover your travel costs. If you withdraw from the study prior to completion (see below) you will receive the vouchers on a pro-rata basis.

**What will happen to the information I provide?**

Your information will be identified by a code so that no data that could personally identify you will be used in any reports. All individual information will be stored securely for 6 years in a locked cabinet and on secure computer networks at the University of Auckland and only the investigators (Assoc Prof Smith, Stefan Wette and Adam Storey) will have access to this information. After the 6 years, all material containing confidential information will be destroyed by shredding. If you wish to gain access to your data, contact the principal researcher and it will be provided to you. Any information that you provide can be disclosed only if (1) it is to protect you or others from harm, (2) a court order is produced, or (3) you provide the researchers with written permission. The results from this study will be used in academic theses, presented at scientific conferences and published in peer-reviewed scientific journals.
Your muscle tissue and blood samples will be stored in a low temperature freezer until analysis is complete. Most samples will be completely destroyed during the analyses. Any remaining samples will be retained until completion of project and thereafter disposed of according to standard biological safety procedures. There will be no opportunity for the return of biological samples at any point throughout the study.

**What are my rights as a participant?**

Participation in this project is voluntary, that is, it is your choice to participate. If you choose to participate you have the right to withdraw from participation in this research (stop taking part) at any time. You have the right to withdraw your data up until 1 July, 2011. You are also encouraged to ask questions if you require clarification about any part of this research project or have any queries as you have the right to have any question answered at any time.

Potential participants who are Māori are encouraged to consult with their whanau, hapu and/or iwi before giving informed consent. Whanau are invited to participate in discussions regarding the research prior to the consent process, and to attend experimental sessions in support of the participant.

**Compensation for Injury**

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation, and Compensation Act 2001. ACC cover is not automatic, and your case will need to be assessed by ACC according to the provisions of the Injury Prevention, Rehabilitation, and Compensation Act 2001. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors, such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses, and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator. You are also advised to check whether participation in this study would affect any indemnity cover you have or are considering, such as medical insurance, life insurance and superannuation.

If you would like further information, please contact ACC (free phone 0800 735566 or website www.acc.co.nz/claims-care/making-a-claim/medical-misadventure/index.html).

**Eligibility**

To participate in this study you must be male, aged 18–35 years and have been performing resistance exercise at least twice per week for the last six months. You must also be of good health, drug-free and not be taking any nutritional supplements. Your eligibility to participate will be based on your responses to the Pre-Participation Questionnaire. You must answer ‘NO’ to all questions to be eligible to participate in this study.

**Further Information**

You will be required to wear suitable sporting clothing and footwear that you feel comfortable exercising in. You will also be required to fill in a food diary.
If you are Interested in Taking Part
Contact:
Associate Professor Heather Smith (PhD)  
Department of Sport and Exercise Science  
University of Auckland, Tamaki Campus  
Auckland, New Zealand  
Phone: +64 9 373 7599 ext 84681  
e-mail: h.smith@auckland.ac.nz

Associate Professor Greg Anson (PhD),  
HOD Department of Sport and Exercise Science  
University of Auckland, Tamaki Campus  
Auckland, New Zealand  
Phone: +64 9 373 7599 ext 82975  
e-mail: g.anson@auckland.ac.nz

Stefan Wette (PGDipSci, MSc candidate)  
Exercise Rehabilitation Practitioner  
The University of Auckland Clinics, Tamaki Campus  
Auckland, New Zealand  
Mobile: 021 032 0535  
e-mail: s.wette@auckland.ac.nz

Chair contact details: For any queries regarding ethical concerns you may contact the Chair, The University of Auckland Human Participants Ethics Committee, The University of Auckland, Office of the Vice Chancellor, Private Bag 92019, Auckland 1142. Telephone 09 373-7599 ext 83711.

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 18/08/2010 for (3) years, Reference Number 2010/314
Interview Questions

Training History

1. How long (months/years) have you been resistance training on a regular basis (at least 2 days/week)?
____________________________________________________________________

2. How often do you perform resistance exercise? __________________________

3. Do you have experience with performing high intensity ‘power’ training including power cleans, snatches and barbell squats?

   Yes/No.

   If yes, please indicate how often? ______________________________

4. Do you perform any other activities other than resistance training (e.g. running, cycling sports)?

   Yes/No.

   If yes, please indicate what and how often you perform these activities?
___________________________________________________________________________

Food

5. Do you have any food allergies (e.g. lactose, gluten etc)?

   Yes/No
If Yes, please provide details?

___________________________________________________________________________
___________________________________________________________________________

Biopsy Safety
To help us ensure your safety please answer the following questions.

6. Have you ever had a negative or allergic reaction to local anaesthesia (e.g. during dental procedures)?
   Yes/No

7. Do you have a tendency toward easy bleeding or bruising (e.g. with minor cuts or shaving)?
   Yes/No

8. Have you ever fainted or do you have any tendency to faint or feel unwell when undergoing or watching medical procedures?
   Yes/No

I confirm that the written information is the same as my verbal answers given during this interview.

Subject signature: ________________________________ Date: ________________________

Signature of Person Conducting Assessment: ________________________________________

Personal details
Age: ________________
Phone: ___________________________ Email: ________________________________________
Emergency contact person/number: ________________________________________________

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 18/08/2010 for (3) years, Reference Number 2010/314
CONSENT FORM – Participant Volunteers

THIS FORM WILL BE HELD FOR A PERIOD OF 6 YEARS

Project title: Molecular responses to high-intensity dynamic resistance (‘power’) exercise and feeding during recovery in resistance trained men

Name(s) of Researcher(s): Assoc Prof Heather Smith, Stefan Wette and Adam Storey

I have read the Participant Information Sheet, have understood the nature of the research and why I have been selected. I have had the opportunity to ask questions and have them answered to my satisfaction.

- I agree to take part in this research.
- I understand that my participation in this research is voluntary.
- I realise that the completion of this study will take approximately 24 hours of my time.
- I understand that I am free to withdraw my participation at any time, and to withdraw any data traceable to me up to 23 December, 2010.
- I do / do not wish to receive data from my maximal strength, power and body size measurements.
- I understand that data will be kept for 6 years, after which it will be destroyed.
- I understand no material that could personally identify me will be used in any reports or presentations of this project.
- I understand that blood samples will be taken and am aware of the possible side effects including bruising and a small possibility of infection.
- I understand the possible side effects of complications associated with the muscle biopsy procedure, including the possibility of (a) a mild bruise like feeling at the sample site the day following the muscle biopsy (b) possible minor bleeding leaving a bruise and pencil-line scarring of the skin at the sample site (c) extremely remote risk of a small decrease in muscle size where the biopsy was taken.
- I understand that I will be required to not perform any intense resistance exercise for two days prior to each experimental trial, and to complete a food diary for two days prior to each experimental trial, and repeat these meals recorded for the two days before each of the subsequent two trials.
- I understand that I will be required to eat the food provided during each experimental trial.

Name ___________________________ Signature __________________________ Date _________________

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 18/08/2010 for (3) years, Reference Number 2010/314
Pre-Participation Questionnaire

Please answer all of the following questions by ticking only one box for each question:
The information provided by you on this form will be treated in the strictest confidence.

1. Do you have any current or previous musculoskeletal injuries that would prevent you from performing a whole body ‘power’ training exercise session?
   Yes ☐  No ☐

2. Do you have any known previous or current heart disease, high blood pressure, diabetes or metabolic conditions, or any other illness?
   Yes ☐  No ☐

3. Do you have a bleeding disorder, past history of bleeding, or are taking any medications that prolong bleeding time or increase the chance of bruising (e.g. anticoagulant therapy, Aspirin, Coumadin, Anti-inflammatories, Plavix)?
   Yes ☐  No ☐

4. Are you free of any illness or infectious disease that may be transmitted in blood?
   Yes ☐  No ☐

5. Do you take any regular medications, regularly use nutritional supplements, or smoke cigarettes?
   Yes ☐  No ☐

6. Are you taking any performance enhancing substances?
   Yes ☐  No ☐

7. Have you been performing resistance exercise for less than 2 days per week for the past 6 months?
   Yes ☐  No ☐

Participant Name (Print): ______________________________________________________

I have read, understood and completed this questionnaire.

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 18/08/2010 for (3) years, Reference Number 2010/314
Care Following Your Muscle Biopsy

Biopsy wound care:
1) Leave outer pressure bandage on for at least 6 hours.
2) Leave the small ‘steri-strip’ biopsy wound covering on for 2-3 days. They will fall off over time while healing occurs.
3) Do not take medications that will thin the blood (e.g., aspirin) for the rest of the day.
4) Watch for trouble signs (listed below) and contact a physician immediately if any appear.
5) Waterproof coverings (Tegaderm) to be placed over top of the steri-strips will be given to you for use whilst showering. They may be left on for several hours before removal (and leaving the steri-strips in place).
6) Additional steri-strips will be given to you; if the strips first applied do not remain secure for 2 days, you can add new strips on top of them using clean hands and avoiding touching or disturbing the healing wound.

Resuming exercise and bathing:
1) You may resume normal daily activities (i.e., walking) after leaving the laboratory after the biopsy.
2) You may resume mild exercise after 24 hours after the biopsy.
3) Vigorous exercise should be avoided for the first 3 days after a muscle biopsy. Avoid direct contact and contact sports until the area is not tender.
4) Daily showers are acceptable, but baths, swimming, saunas etc. should be avoided for at least 4 days following a biopsy.
5) Waterproof coverings (Tegaderm) to be placed over top of the steri-strips will be given to you for use whilst showering. They may be left on for several hours before removal (and leaving the steri-strips in place).
Trouble signs: If you notice any of these signs after your biopsy, you should contact a physician immediately.

- Redness, excessive swelling, tenderness, or increased warmth of the skin around the biopsy wound.
- Throbbing pain or tenderness in the biopsy wound area.
- Red streaks in the skin around or progressing away from the biopsy wound.
- Pus or watery discharge collected beneath the skin or draining from the wound.
- Foul odor from the wound.
- Tender lumps or swelling in your armpit, groin, or neck.
- Generalized chills or fever.

If you have any questions or concerns, contact the biopsy physician or A/Prof Smith:
A/Prof Heather Smith: Tel. 373-7599 ext 84681 or 021 141 2461, h.smith@auckland.ac.nz

Physician: Tel. __________________, __________________, ________________________________

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 18/08/2010 for (3) years, Reference Number 2010/314
Body Composition Measurements:

The standard nine skin-fold measurements sites used in Study 3. Photographs are adapted from Heyward (2002).

CHEST

Landmark and Pinch

- The pinch is taken at a point between the axilla and nipple as high as possible on the anterior axillary fold.

BICEP

Landmark and Pinch

- A mark is made at the level of the mid-point between the acromiale and the radiale on the mid-line of the anterior surface of the arm (over the biceps muscle).
- The arm should be relaxed with the palm of the hand facing forwards.
- A vertical pinch, parallel to the long axis of the arm, is made at the landmark.
TRICEPS

Landmark and Pinch

- A mark is made at the level of the mid-point between the acromiale and the radiale on the mid-line of the posterior (back) surface of the arm (over the triceps muscle).
- The arm should be relaxed with the palm of the hand facing forwards (supinated).
- A vertical pinch, parallel to the long axis of the arm, is made at the landmark.

SUBSCAPULAR

Landmark and Pinch

- A mark is made at the lower angle of the scapula (bottom point of shoulder blade).
- The pinch is made following the natural fold of the skin, approximately on a line running laterally (away from the body) and downwards (at about 45 degrees).
ABDOMEN

Landmark and Pinch

- A mark is made 5 cm to the right side of the umbilicus.
- The vertical pinch is made at the marked site, and the callipers placed just below the pinch.

SUPRAILIAC

Landmark and Pinch

- A mark is made immediately above the iliac crest, on the most lateral aspect.
- The fold is directed anteriorly and downward in line with the natural fold of the skin. The right arm should be held across the body to keep it away from the measurement area.
SUPRASPINALE

Landmark and Pinch

- A mark is made at the intersection of a line joining the spinale and the anterior part of the axilla, and a horizontal line at the level of the iliac crest.
- The pinch is directed medially and downward, following the natural fold of the skin (at an approximate angle of 45 degrees).

CALF

Landmark and Pinch

- A mark is made on the medial surface of the calf, at the level of the largest circumference.
- The subject should place their knee at approximately 90 degrees and a vertical pinch parallel to the long axis of the leg is taken.
FRONT THIGH

Landmark and Pinch

- A mark is made at the mid-point of the anterior surface of the thigh, midway between patella and inguinal fold.
- A vertical pinch is taken. This measurement is normally taken with the subject sitting and the knee bent at right angles.

Equations used to calculate body composition:

Body density was estimated as per the method of Jackson et al. (1978):

\[
\text{Body Density} = 1.10938 - (0.0008267 \times \text{sum of chest, abdomen and thigh skin-folds in mm}) + \\
(0.0000016 \times \text{square of the sum of chest, abdomen and thigh}) - (0.0002574 \times \text{age})
\]

Body fat was estimated as per the method of Siri (1978):

\[
\text{Percentage Body Fat} = (495 \div \text{Body Density}) - 450.
\]
Table B-1: Standardized meal components.

Study 1:

### Subway® Wheat Bread (78g Serving Size)

<table>
<thead>
<tr>
<th>Component</th>
<th>Total per Serving (g)</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>8.0</td>
<td>Wheat flour, water, yeast, wheat bran, sugar, wheat gluten, iodised salt, canola oil, palm oil, soya flour, emulsifiers (481, 472e, 471), malt flour, caramel (150c), food acid (300), enzymes, anti-caking agent (535), thiamine, folic acid.</td>
</tr>
<tr>
<td>Fat</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>Na2+</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td><strong>Total Weight (g)</strong></td>
<td><strong>50.3</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total Energy (kJ)</strong></td>
<td><strong>879.0</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Subway® Chicken Breast Strips (71g Serving Size)

<table>
<thead>
<tr>
<th>Component</th>
<th>Total per Serving (g)</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>16.0</td>
<td>Chicken (94%), soy protein, salt, modified starch (1412), flavour [hydrolysed vegetable protein (Ubogu et al.)], mineral salt (450), vegetable oil [canola (antioxidant (319))].</td>
</tr>
<tr>
<td>Fat</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Na2+</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Total Weight (g)</strong></td>
<td><strong>17.7</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total Energy (kJ)</strong></td>
<td><strong>335.0</strong></td>
<td></td>
</tr>
</tbody>
</table>
### Study 3

**One Square Meal Bar (85g net/bar)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Total per Serving (g)</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>16.70</td>
<td>Rolled oats, brown rice syrup, vegetable oil, maltodextrin, isolated soy protein (non GM), dried cranberries 6% (cranberries, deionised &amp; clarified pineapple juice concentrates, vegetable oil), whole grain wheat, humectant (glycerin), rice flour, vegetable gum (acacia), raisins, Manuka honey 2%, blackcurrant fruit pieces 2% (fruit (concentrated apple puree, deionised apple juice concentrate, blackcurrant puree, concentrated blackcurrant juice), wheat fibre, gelling agent (pectin) natural flavour, emulsifier (soy lecithin), salt, vitamins &amp; minerals (calcium, magnesium, vitamin C, niacin, vitamin E, zinc, vitamin B2, vitamin B6, iron, vitamin B1, vitamin A, folate, iodine, vitamin D, vitamin B12), natural flavour, food acid (citric acid), malted barley extract. Contains soy &amp; gluten containing cereal products. This product or some of its ingredients are made on equipment that handles sesame seeds, tree nuts &amp; milk products.</td>
</tr>
<tr>
<td>Fat</td>
<td>23.30</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>90.20</td>
<td></td>
</tr>
<tr>
<td>Na2+</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Minerals</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Dietary Fibre</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td>Total Weight (g)</td>
<td>141.45</td>
<td></td>
</tr>
<tr>
<td>Total Energy (kJ)</td>
<td>2905.64</td>
<td></td>
</tr>
</tbody>
</table>

**Meadow Fresh Chocolate Milk (600 ml)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Total per Serving (g)</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>25.80</td>
<td>1.7% fat milk, milk solids, cocoa, (0.5%), chocolate flavour, vitamins A &amp; D, stabiliser 407</td>
</tr>
<tr>
<td>Fat</td>
<td>10.20</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>57.60</td>
<td></td>
</tr>
<tr>
<td>Na2+</td>
<td>0.00024</td>
<td></td>
</tr>
<tr>
<td>Ca2+</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.000003</td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.00033</td>
<td></td>
</tr>
<tr>
<td>Total Weight (g)</td>
<td>94.56</td>
<td></td>
</tr>
<tr>
<td>Total Energy (kJ)</td>
<td>1800.00</td>
<td></td>
</tr>
</tbody>
</table>
## Appendix C: Research Questionnaires

### Daily Analysis of Life Demands of Athletes (DALDA)

**Name:**

**Date:**

**Directions:**
RESPOND BY CIRCLING the appropriate response alongside each item.
If you are unsure of an item, please see the attached definitions section.
A = Worse than Normal;  B = Normal;  C = Better than Normal

### PART A

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Home-Life</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>School/College/Work</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>Friends</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>Sport Training</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>Climate</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>Sleep</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>Recreation</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>Health</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
</tbody>
</table>

### TOTAL RESPONSES

### PART B

<table>
<thead>
<tr>
<th></th>
<th>Muscle Pains</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Techniques</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>Tiredness</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>Need For A Rest</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>Supplementary Work</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>Boredom</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>Recovery Time</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>Irritability</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>Weight</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>10</td>
<td>Throat</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>11</td>
<td>Internal</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>12</td>
<td>Unexplained Aches</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>13</td>
<td>Technique Strength</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>14</td>
<td>Enough Sleep</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>15</td>
<td>Between Sessions Recovery</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>16</td>
<td>General Weakness</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>17</td>
<td>Interest</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
</tbody>
</table>
## DAILY ANALYSIS OF LIFE DEMANDS OF ATHLETES DEFINITIONS

### PART A: Sources of Life Stress

<table>
<thead>
<tr>
<th></th>
<th>Sources</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Consider whether you are eating regularly and in adequate amounts. Are you missing meals? Do you like your meals?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Home Life</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Have you had arguments with your parents, brother, or sisters? Are you being asked to do too much around the house? How is your relationship with your wife/husband? Have there been any unusual happenings at home concerning your family?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>School/College/Work</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Consider the amount of work you are doing there. Are you required to do more or less at home or in your own time? How are you grades or evaluations? Think of how you are interacting with administrators, teachers, or bosses.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Friends</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Have you lost or gained any friends? Have there been any arguments or problems with your friends? Are they complimenting your more or less? Do you spend more or less time with them?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Training and Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>How much and how often are you training? Are the levels of effort required easy or hard? Are you able to recovery adequately between efforts? Are you enjoying your sport?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Climate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Is it too hot, cold, wet, or dry?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sleep</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Are you getting enough sleep? Are you getting too much? Can you sleep when you want too?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Recreation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Consider the activities you are doing outside of your sport. Are they taking up too much time? Do they complete with your application to your sport?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Health</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Do you have any infections, a cold, or other temporary health problems?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PART B: Symptoms of Stress

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Muscle Pains</td>
<td>Do you have sore joints and/or pains in your muscles</td>
</tr>
<tr>
<td>2</td>
<td>Techniques</td>
<td>How do your techniques feel?</td>
</tr>
<tr>
<td>3</td>
<td>Tiredness</td>
<td>What is your general state of tiredness</td>
</tr>
<tr>
<td>4</td>
<td>Need For a Rest</td>
<td>Do you feel like you need a rest between training sessions?</td>
</tr>
<tr>
<td>5</td>
<td>Supplementary Work</td>
<td>How strong do you feel when you do supplementary training (i.e. weights, resistance work, stretching)?</td>
</tr>
<tr>
<td>6</td>
<td>Boredom</td>
<td>How boring is training?</td>
</tr>
<tr>
<td>7</td>
<td>Recovery Time</td>
<td>Do your recovery times between each training effort need to be longer?</td>
</tr>
<tr>
<td>8</td>
<td>Irritability</td>
<td>Are you irritable? Do things get on your nerves?</td>
</tr>
<tr>
<td>9</td>
<td>Weight</td>
<td>How is your weight?</td>
</tr>
<tr>
<td>10</td>
<td>Throat</td>
<td>Have you noticed your throat being sore or irritated?</td>
</tr>
<tr>
<td>11</td>
<td>Internal</td>
<td>How do you feel internally? Have you constipation, upset stomachs, etc.?</td>
</tr>
<tr>
<td>12</td>
<td>Unexplained Aches</td>
<td>Do you have any unexplained aches or pains?</td>
</tr>
<tr>
<td>13</td>
<td>Technique Strength</td>
<td>How strong do your techniques feel?</td>
</tr>
<tr>
<td>14</td>
<td>Enough Sleep</td>
<td>Are you getting enough sleep?</td>
</tr>
<tr>
<td>15</td>
<td>Between Sessions Recovery</td>
<td>Are you tired before you start your second training session of the day?</td>
</tr>
<tr>
<td>16</td>
<td>General Weakness</td>
<td>Do you feel weak all over?</td>
</tr>
<tr>
<td>17</td>
<td>Interests</td>
<td>Do you feel you are maintaining your interest in your sport?</td>
</tr>
<tr>
<td>18</td>
<td>Arguments</td>
<td>Are you having squabbles and arguments with people?</td>
</tr>
<tr>
<td>19</td>
<td>Skin Rashes</td>
<td>Do you have any unexplained skin rashes or irritations?</td>
</tr>
<tr>
<td>20</td>
<td>Congestion</td>
<td>Are you experiencing congestion in the nose and/or sinuses?</td>
</tr>
<tr>
<td>21</td>
<td>Training Effort</td>
<td>Do you feel you can give your best effort at training?</td>
</tr>
<tr>
<td>22</td>
<td>Temper</td>
<td>Do you lose your temper?</td>
</tr>
<tr>
<td>23</td>
<td>Swellings</td>
<td>Do you have any lymph gland swellings under your arms, below your ears, in your groin, etc.?</td>
</tr>
<tr>
<td>24</td>
<td>Likability</td>
<td>Do people seem to like you?</td>
</tr>
<tr>
<td>25</td>
<td>Running Nose</td>
<td>Do you have a running nose?</td>
</tr>
</tbody>
</table>

Profile of Mood States Questionnaire (POMS)

Name: ____________________________

Date: ____________________________

Directions: Describe HOW YOU FEEL RIGHT NOW by circling the appropriate number for each of the words listed below.

<table>
<thead>
<tr>
<th>Feeling</th>
<th>Not At All</th>
<th>A Little</th>
<th>Moderate</th>
<th>Quite a Bit</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friendly</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Tense</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Angry</td>
<td>1</td>
<td>2</td>
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<td>4</td>
<td>5</td>
</tr>
<tr>
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<td>5</td>
</tr>
<tr>
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<td>2</td>
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<td>4</td>
<td>5</td>
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<td>Clear-Headed</td>
<td>1</td>
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<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
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<td>2</td>
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<td>5</td>
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<tr>
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<td>Not At All</td>
<td>A Little</td>
<td>Moderate</td>
<td>Quite a Bit</td>
<td>Extremely</td>
</tr>
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<td>4</td>
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<td>4</td>
<td>5</td>
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<td>4</td>
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<td>4</td>
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<td>5</td>
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<td>Guilty</td>
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<td>5</td>
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<tr>
<td>Uncertain About Things</td>
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<td>4</td>
<td>5</td>
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<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Profile of Mood States Questionnaire developed by:
Two-day Dietary Record

This booklet is used to record your daily consumption of food over two days prior to each experimental trial (repeat record 3 times). Please be aware that the information gained from your dietary record is critically dependent on the accuracy of the data you record. Please provide an honest recall of the foods and drink consumed throughout the day. You will be complete a dietary record food diary for two days prior to each experimental trial, and repeat these meals recorded for the two days before each of the subsequent two trials. Therefore, we recommend you consume foods that can be easily replicated.

Recording Instructions

Date / Time
- List the date and time you consumed the food or beverage

Food / Beverage
- Write the name of the food or beverage (include brand names if from a packet)
- Remember to list all additions to foods such as sugar, butter, salad dressing etc.

Amount
List the approximate amount eaten or drunk in milliliters (ml), grams (gm) or in units such as 1 slice whole wheat bread or 1 apple etc.
- In mixed food dishes, for example a stir fry, provide approximations (where possible) of how much of each food source there is in the dish i.e. one handful of beans, one breast of chicken, one cup (250ml) mixed vegetables etc.
- As an alternative you may provide a copy of the recipe and then list what proportion of the entire recipe you ate.

Description / Preparation
- Describe the methods used to prepare the food or beverage. For example baked, fried, grilled, steamed, raw, with sauce etc.
Supplements

- Nutritional or performance enhancing supplements (protein powders, creatine etc) are not permitted during this study.

**EXAMPLE TEMPLATE:**

**EXPERIMENTAL TRIAL 1**

**NUTRITION DIARY - DAY ONE**

<table>
<thead>
<tr>
<th>Time</th>
<th>Food / Beverage / Supplement</th>
<th>Amount</th>
<th>Description/Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Name: __________________________ Date: __________________________
Rating of Perceived Exertion (RPE) Scale

This is a scale for rating perceived exertion. Perceived exertion is the overall effort or distress of your body during exercise.

- If you have a RPE of 6, you would be sitting doing nothing.
- If you have a RPE of 12, you could continue with your activity, at the same intensity, for another 45 min.
- If you have a RPE of 15, you could continue with your activity, at the same intensity, for another 30 min.
- If you have a RPE of 18, you could continue with your activity, at the same intensity, for another 5 min.
- An RPE of 20 represents the greatest amount of exertion that you are able to achieve – and you cannot continue.

<table>
<thead>
<tr>
<th>RPE</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Nothing at all</td>
</tr>
<tr>
<td>7</td>
<td>Very, very light</td>
</tr>
<tr>
<td>8</td>
<td>Just noticeable</td>
</tr>
<tr>
<td>9</td>
<td>Very light</td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Fairly light</td>
</tr>
<tr>
<td>12</td>
<td>Light</td>
</tr>
<tr>
<td>13</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>14</td>
<td>Hard</td>
</tr>
<tr>
<td>15</td>
<td>Heavy</td>
</tr>
<tr>
<td>16</td>
<td>Very hard</td>
</tr>
<tr>
<td>17</td>
<td>Very, very hard</td>
</tr>
<tr>
<td>18</td>
<td>Strongest intensity</td>
</tr>
</tbody>
</table>

## 9.4 Appendix D: Study 1 Training Program Format

The following tables depict the number of sets and repetitions performed at the target intensity for weightlifting-specific exercises. It does not include a description of the preceding sets and repetitions performed at lower intensities or the supplementary exercises (e.g., presses and abdominal work) that were performed. The target intensities for each exercise were reached within 4-7 sets. The repetition range for the preceding sets ranged from 1-5 repetitions.

### Table D-1: Study 1 training program overview.

<table>
<thead>
<tr>
<th>Week 1 - Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monday AM</strong></td>
</tr>
<tr>
<td>Exercise</td>
</tr>
<tr>
<td>Snatch</td>
</tr>
<tr>
<td>C&amp;J</td>
</tr>
<tr>
<td>Fr Squat</td>
</tr>
<tr>
<td>Bk Squat</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Monday PM</strong></th>
<th><strong>Tuesday PM</strong></th>
<th><strong>Wednesday PM</strong></th>
<th><strong>Thursday PM</strong></th>
<th><strong>Friday PM</strong></th>
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</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>% 1RM</td>
<td>Volume</td>
<td>Exercise</td>
<td>% 1RM</td>
<td>Volume</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C&amp;J</td>
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<td>Bk Squat</td>
<td>85</td>
<td>2x1</td>
<td>Fr Squat</td>
<td>85</td>
<td>2x1</td>
</tr>
</tbody>
</table>

C&J = Clean and Jerk; Fr Squat = Front Squat; Bk Squat = Back Squat; Pow Sn = Power Snatch; Pow Cl = Power Clean; VJ = Vertical Jump
**Table D-1: Continued.**

### Week 2 - Overload

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<th>%1RM</th>
<th>Volume</th>
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<th>Volume</th>
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<th>Volume</th>
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<td>Pow Sn</td>
<td>92</td>
<td>2x1</td>
<td>C&amp;J</td>
<td>90</td>
<td>1x1</td>
<td>Pow Sn</td>
<td>92</td>
<td>1x1</td>
<td>VJ Testing</td>
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</tr>
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<td>C&amp;J</td>
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<td>Sn Pull</td>
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<td>Fr Squat</td>
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<td>Pow Cl</td>
<td>92</td>
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<td>Pre-Ex Blood</td>
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<td>Cl Pull</td>
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<td>C&amp;J</td>
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<td>2x1</td>
<td>Post-Ex Blood (0min)</td>
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<td>C&amp;J</td>
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Sn Pull = Snatch Pull; Cl Pull = Clean Pull
Table D-1: Continued.

Week 3 - Overload

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#### Week 4 - Recovery

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9.5 Appendix E: Exercise Instructions

STUDY 3:

Power Snatch from Hang

1. Using a wide overhand or hook grip, begin with a loaded barbell from a rack set at a mid-thigh height and step forward with the loaded barbell.
2. The feet should be positioned directly below the hips, with the feet slightly turned out.
3. Slightly bend at the knees and incline the torso forward to allow the barbell to slide down the thighs to a starting position that is just above knee level. At this position, the spine should be extended, the shoulders must remain over the barbell, the elbows point outwards and the head is facing forward.
4. Initiate the movement by driving through the heels and extending the back whilst maintaining the shoulder position over the barbell.
5. Finish off the peak extension by a synchronistic shrugging of the shoulders and ballistic extension of the hips.
6. Allow the elbows to flex to enable the barbell to rise in the vertical plane. It is essential to keep the barbell as close to the body as possible.
7. Rapidly move your feet into the receiving position as you forcefully pull your body below the barbell to adopt the overhead receiving position with arms fully extended.
8. A power snatch requires the barbell to be received in an overhead position that is above a parallel squat.
9. Recover from the semi-squat position whilst maintaining the barbell overhead.

TIPS:

1. In the overhead position, the barbell should be position behind the ears and the weight of the barbell should be centred over the lifter’s centre of mass.
2. Avoid jumping forward to receive the barbell.
3. In the starting position, don’t allow the shoulders to roll forward.
Power Clean (1) / Front Squat (2) / Push Press (3)

1. Begin with a loaded barbell on the ground.
2. The barbell should be set close to the shins and a wide overhand or hook grip should be taken.
3. The feet should be positioned directly below the hips, with the feet slightly turned out.
4. The starting position will require the lifter to lower their hips, elevate the chest, point the elbows outwards and direct the eye line slightly upwards. The shoulders must remain over the barbell in this set position.
5. Initiate the movement by driving through the heels and extending the back whilst maintaining the shoulder position over the barbell.
6. Finish off the peak extension by a synchronistic shrugging of the shoulders and ballistic extension of the hips.
7. As the barbell rises, rapidly move your feet into the receiving position as you forcefully pull your body under the barbell.
8. Thrust your elbows forward to allow the barbell to be received on the front of the shoulders in a semi-squat position.
9. Recover from the semi-squat position whilst maintaining the barbell on top of the shoulders.
10. Bend at the knees into a full squat position whilst maintaining the correct elbow and head position.
11. Recover from the full squat position whilst exhaling on the exertion.
12. Whilst keeping the torso vertical, flex at the into a quarter squat.
13. At the lowest point of the dip, forcefully drive through the heels to allow the barbell to rise in the vertical plane.
14. Using the momentum created by your leg drive, continue the movement by pressing the barbell overhead into a fully extended arm position.
15. Lower the barbell to the starting position in a controlled fashion.
Box Jumps

1. **WARM UP:** Stand with feet shoulder width apart and perform 3 counter-movement jumps onto an 80 cm high wooden box.
2. **PROTOCOL:** Stand with feet shoulder width apart and perform a counter-movement jump onto a 1 metre high wooden box.
3. Perform the concentric portion of the jump in an ‘explosive’ fashion.
4. Land with both feet on top of the box in a semi squat position to absorb the downward forces.
5. Step down from the box and repeat for 3 repetitions per set.

Hurdle Jumps

1. **SET UP:** Set out four 0.75 m high hurdles 0.7 m apart from each other.
2. **PROTOCOL:** Stand with feet shoulder width apart and perform successive two-legged counter-movement jumps over each hurdle.
3. Aim to minimize the ground contact time between hurdles and perform the concentric portion of the jump in an ‘explosive’ fashion.
4. After completing four hurdle jumps, walk back to the start to repeat the number of repetitions (12 hurdle jumps per set).

Single-Legged Triple Jumps

1. **SET UP:** Markers are placed on a flat non-slip surface approximately 10 m apart.
2. **PROTOCOL:** Participants aim to achieve the target distance of ~20 m in a total of 4 jumps (2 per leg) as outlined below. Two repetitions/lengths (~20 m each) are to be completed per set with a 30sec rest interval between repetitions/lengths.

3. Participants were required to complete 4 sets (i.e. 8 x ~20 m lengths) for this exercise.

1. Right foot hop
2. Step onto left foot hop
3. Land on both feet
4. Turn around and repeat the sequence, starting with the opposite leg to complete 1 length (~20m)
9.6 Appendix F: International Weightlifting Federation Technical Rules for the Snatch and Clean and Jerk

2009 - 2012 International Weightlifting Federation Technical and Competition Rules (Section 2).

2.1 THE SNATCH

2.1.1 The barbell is placed horizontally in front of the lifter’s legs. It is gripped, palms downwards and pulled in a single movement from the platform to the full extent of both arms above the head, while either splitting or bending the legs. During this continuous movement, the barbell may slide along the thighs and the lap. No part of the body other than the feet may touch the platform during the execution of the lift. The weight, which has been lifted, must be maintained in the final motionless position, arms and legs extended, the feet on the same line, until the Referees give the signal to replace the barbell on the platform. The lifter may recover in his or her own time, either from a split or a squat position, and finish with the feet on the same line, parallel to the plane of the trunk and the barbell. The Referees give the signal to lower the barbell as soon as the lifter becomes motionless in all parts of the body.

2.2 THE CLEAN AND JERK

2.2.1 The first part, the Clean:
The barbell is placed horizontally in front of the lifter’s legs. It is gripped, palms downwards and pulled in a single movement from the platform to the shoulders, while either splitting or bending the legs. During this continuous movement, the barbell may slide along the thighs and the lap. The barbell must not touch the chest before the final position. It then rests on the clavicles or on the chest above the nipples or on the arms fully bent. The feet return to the same line, legs straight before performing the Jerk. The lifter may make this recovery in his or her own time and finish with the feet on the same line, parallel to the plane of the trunk and the barbell.
2.2.2 **The second part, the Jerk:**

The athlete bends the legs and extends them as well as the arms to bring the barbell to the full stretch of the arms vertically extended. He or she returns the feet to the same line; arms and legs fully extended, and waits for the Referees’ signal to replace the barbell on the platform. The Referees give the signal to lower the barbell as soon as the lifter becomes motionless in all parts of the body.

**IMPORTANT REMARK:**

After the Clean and before the Jerk, the lifter may adjust the position of the barbell. This must not lead to confusion. It does not mean the granting of an additional jerk attempt but allowing the lifter to:

A) Withdraw the thumbs or “unhook” if this method is used,
B) Lower the barbell in order to let it rest on the shoulders if the barbell is placed too high and impedes the breathing or causes pain,
C) Change the width of the grip.

**2.3 GENERAL RULES FOR ALL LIFTS**

2.3.1 The technique known as “hooking” is permitted. It consists of covering the last joint of the thumb with the other fingers of the same hand at the moment of gripping the barbell.

2.3.2 In all lifts, the Referees must count as “No lift” any unfinished attempt in which the barbell has reached the height of the knees.

2.3.3 After the Referees signal to lower the barbell, the lifter must lower it in front of the body and not let it drop either deliberately or accidentally. The grip on the barbell may be released when it has passed the level of the shoulders.

2.3.4 A competitor, who cannot fully extend the elbow due to an anatomical deformation, must report this fact to the three Referees and the Jury before the start of the competition.

2.3.5 When snatching or cleaning in the squat style, the lifter may help the recovery by making swinging and rocking movements of the body.

2.3.6 The use of grease, oil, water, talcum or any similar lubricant on the thighs is forbidden. Lifters are not permitted to have any substance on their thighs when arriving in the
competition area. A lifter who uses any lubricant is ordered to remove it. During the removal the clock goes on.

2.3.7 The use of chalk (magnesium) on the hands, thighs, etc., is permitted.

2.4 INCORRECT MOVEMENTS AND POSITIONS FOR ALL LIFTS

2.4.1 Pulling from the hang.
2.4.2 Touching the platform with any part of the body other than the feet.
2.4.3 Uneven or incomplete extension of the arms, at the finish of the lift.
2.4.4 Pause during the extension of the arms.
2.4.5 Finishing with a press-out.
2.4.6 Bending and extending the elbows during the recovery.
2.4.7 Leaving the platform during the execution of the lift, i.e. touching the area outside the platform with any part of the body.
2.4.8 Replacing the barbell on the platform before the Referees’ signal.
2.4.9 Dropping the barbell after the Referees’ signal.
2.4.10 Failing to finish with the feet and the barbell in line and parallel to the plane of the trunk.
2.4.11 Failing to replace the complete barbell on the platform, i.e. the complete barbell must first touch the platform.
2.4.12 Not facing the Centre Referee at the beginning of a lift.

2.5 INCORRECT MOVEMENTS FOR THE SNATCH

2.5.1 Pause during the lifting of the barbell.
2.5.2 Touching the head of the lifter with the bar.

2.6 INCORRECT MOVEMENTS FOR THE CLEAN

2.6.1 Placing the bar on the chest before turning the elbows.
2.6.2 Touching the thighs or the knees with the elbows or the upper arms.
2.7 INCORRECT MOVEMENTS FOR THE JERK

2.7.1 Any apparent effort of jerking which is not completed. This includes lowering the body or bending the knees.

2.7.2 Any deliberate oscillation of the barbell to gain advantage. The athlete and the barbell have to become motionless before starting the jerk.
9.7 Appendix G: Experimental Solutions

Study 1:

**PBS Washing Buffer (0.1M PBS) - pH 7.4**

**10x Stock Solution (1L)**

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<td>KCl</td>
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<td>Na$_2$HPO$_4$.7H$_2$O</td>
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<td>1.4mM</td>
<td>KH$_2$PO$_4$</td>
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d H$_2$O (MiliQ) to 1 litre

**1x Stock Solution**

1 part 10x Stock Solution, 9 parts d H$_2$O (MiliQ) to 1 Litre

**BioCell Protein Carbonyl Test Kit (Batch: P096)**

**Pre-Assay Preparation:**

1) **EIA Buffer (C).**

The contents of bottle C was dissolved in one litre of deionized water.

2) **Blocking reagent (D).**

2ml of EIA Buffer was added to the blocking reagent container (D). The contents were mixed well and transferred to a 100 mL measuring cylinder. Additional EIA Buffer was added to bring the final volume to 75 mL.

3) **Dinitrophenylhydrazine (DNP) (E).**

1 ml of the DNP reagent (E) was added to 9 mL of guanidine hydrochloride (F).

4) **Anti-DNP-biotin-antibody (G).**
The Anti-DNP-biotin-antibody was stored at -20°C until needed. To prepare this dilution, 0.5 mL of “diluted blocking solution” was added to the Anti-DNP-biotin-antibody microvial (G). The contents were mixed well “diluted blocking solution” was added to bring the final volume to 20 mL.

5) Streptavidin-horseradish-peroxidase (H).
Streptavidin-HRP microvial (H) was stored at 4°C until needed. To prepare this dilution, 0.5 mL of “diluted blocking solution” was added to the Streptavidin-HRP microvial (H). The contents were mixed well “diluted blocking solution” was added to bring the final volume to 20 mL.

Quantikine Human MIP-1β Kit (Catalogue Number: DMB00)
Pre-Assay Preparation:

1) Wash Buffer
20 mL of Wash Buffer Concentrate was added into 480 mL of deionized water.

2) Substrate Solution
Colour Reagents A and B were mixed together in equal volumes within 15 minutes of use.

3) MIP-1β Standard
The MIP-1β Standard was reconstituted with 5 mL of Calibrator Diluent RD6O. This reconstitution produces a stock solution of 2000 pg/mL. The reconstituted standard was left to sit for ~15 minutes with gentle agitation prior to making dilutions.

For the dilutions, 500 µL of Calibrator Diluent RD6O was pipette into each tube. The stock solution was used to produce the dilution series below. The undiluted standard served as the highest standard (2000 pg/mL). Calibrator Diluent RD6O served as the zero standard (0 pg/mL).
Study 3:

**ALPCO Cortisol Kit (Catalogue Number: 11-CORHU-E01)**

Pre-Assay Preparation:

1) **Cortisol-Horseradish Peroxidase (HRP) Conjugate Concentrate**
120 µL of HRP was diluted in 12 mL of assay buffer (1:100) before use.

2) **Wash Buffer Concentrate**
50 mL of the wash buffer concentrate was diluted in 450 mL of distilled water.

**Skeletal Muscle Homogenizing/Lysis Buffer**

*Table G-1: Low salt lysis buffer.*

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<td>1 M KCl (mL)</td>
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**Invitrogen Human MCP-1 ELISA Kit (Catalogue Number: KHC1011)**

Pre-Assay Preparation:

1) **Hu MCP-1 Standard**
The Hu MCP-1 standard was reconstituted with 3.1 mL Standard Diluent Buffer to create a final concentration of 10,000 pg/mL. The contents was mixed and allowed to sit for 10 min to ensure complete reconstitution. Serial dilutions of the standard were made as described in Table G13.
Table G-2: Serial dilution of Hu MCP-1 Standard.

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<td>Prepared as above</td>
<td>0.300 mL of the Diluent</td>
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<tr>
<td>500 pg/mL</td>
<td>0.300 mL of the 1000 pg/mL standard</td>
<td>Buffer</td>
</tr>
<tr>
<td>250 pg/mL</td>
<td>0.300 mL of the 500 pg/mL standard</td>
<td>0.300 mL of the Diluent</td>
</tr>
<tr>
<td>125 pg/mL</td>
<td>0.300 mL of the 250 pg/mL standard</td>
<td>Buffer</td>
</tr>
<tr>
<td>62.5 pg/mL</td>
<td>0.300 mL of the 125 pg/mL standard</td>
<td>0.300 mL of the Diluent</td>
</tr>
<tr>
<td>31.2 pg/mL</td>
<td>0.300 mL of the 62.5 pg/mL standard</td>
<td>Buffer</td>
</tr>
<tr>
<td>15.6 pg/mL</td>
<td>0.300 mL of the 31.2 pg/mL standard</td>
<td>0.300 mL of the Diluent</td>
</tr>
<tr>
<td>0 pg/mL</td>
<td>0.300 mL of the Diluent Buffer</td>
<td>An empty tube</td>
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</table>

1) **Final Dilution of Streptavidin-HRP**
For each of the 8 well strips used in the assay, 10 µL of the 100X Streptavidin-HRP concentrated solution was diluted with 1 mL of Streptavidin HRP Diluent.

2) **Wash Buffer**
The Wash Buffer Concentrate (25X) was brought to room temperature and mixed to ensure that any precipitated salts were dissolved. 1 volume of the Wash Buffer Concentrate (25X) was diluted with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 litres, 100 mL may be diluted up to 2.5 litres).
mRNA Quantity and Quality Checks: Study 1 and Study 3

**Figure H-1:** The concentration and purity of total RNA in diluted samples (1:10 vol/vol with RNase-free water) was determined using a NanoDrop 1000 spectrophotometer (ND-1000, Thermo Fisher Scientific, Wilmington DE, USA). The 260/280 absorbance ratio was used to assess the purity of total RNA; a ratio of ~2.0 is generally accepted as “pure” for total RNA. In the current investigation, all samples used for further analysis were deemed to be of acceptable purity with a mean (± SD) 260/280 ratio of 1.91 ± 0.11.
Figure H-2: The quality of the total RNA was determined on an Agilent 2100 Bioanalyzer® (Agilent Technologies, Palo Alto, CA) and samples were run on RNA 6000 Nano LabChips. The Bioanalyzer software automatically calculates the ratio of the 18S to 28S ribosomal subunits and generates an RNA integrity number (RIN) with 1 being the most degraded and 10 being the most intact. In the current investigation, all samples used for further analysis were deemed to be of acceptable purity with a mean (± SD) RIN of 8.78 ± 2.63.
cDNA Microarray Quality Control Metrics: Study 1

Pseudo-Coloured Arrays
Figure H-3: Pseudo-coloured arrays were generated with the pseudo palette function (affyPLM library) to help identify possible artefacts and/or differences in intensities within and between the arrays. Following the hydrolysis of cRNA and the cleanup of single-stranded DNA, it was noted that Array 2 (AS0002) had a low cDNA yield which is evident in the “lighter” appearance of the array.
Figure H-4: Raw array signal intensities prior to performing the Robust Multiarray Average (RMA) algorithm correction. Array 2 has a smaller interquartile range most likely due to the low cDNA yield and Array 8 has a larger distribution tail most likely due to the artifact evident on the pseudo-coloured array.

Figure H-5: Raw array signal intensities after performing the Robust Multiarray Average (RMA) algorithm correction.
Figure H-6: Raw array log \textsubscript{2} probe hybridisation signal intensities prior to performing the Robust Multiarray Average (RMA) algorithm correction. The signal intensity for Array 2 clearly differs and the small “bump” for Array 8 is likely due to the array artefact.

Figure H-7: Raw array log \textsubscript{2} probe hybridisation signal intensities after performing the Robust Multiarray Average (RMA) algorithm correction. Array 2 remained to be problematic.
Figure H-8: The MvA plots showing the difference in the log intensities (M), and average log intensity (A). If two well-prepared arrays with identical treatments were compared, it would be expected that the graph would show tight clustering about M=0 for all average intensities. Array 2 (AS0002) clearly shows a different MvA plot due to the low cDNA yield prior to hybridisation.
Figure H-9: Example qPCR amplification plot and Ct determination for the gene of interest, Chemokine (C-C motif) Receptor 2 (CCR2) (Hs00356601_m1).
Figure H-10: An example force trace used to determine peak force, contractile rate of force development, peak rate of force development (Study 2 and 3) and contractile impulse (Study 2). The onset of contraction was defined as the time point on the force-time curve where force exceeded the baseline by > 7.5N (Aagaard et al., 2002).
Figure H-11: An example force trace that was excluded from analysis due to the negative deflection which was indicative of a counter-movement.
Figure H-12: Example force traces used to determine; 1) static jump and, 2) counter-movement vertical jumps (CMJ) height. For each trial, “flight-time” (C) was determined as the duration between the takeoff (A) and impact (B) and was used to calculate the maximum rise in the participant’s centre of gravity.
Equations used to calculate maximum jump height (cm)

**Vertical Velocity** ($V_v$)

$$V_v = \frac{1}{2} \times t_{air} \times g$$

$g$ = acceleration of gravity (9.81 m/s$^2$)

**Maximum Jump Height** ($h$)

$$h = \frac{V_v^2}{2 \times g}$$
10.0 REFERENCES


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