The effect of concurrent resistance and repeated-sprint exercise on performance and the cytokine response in female team-sport athletes

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In memory of Dr Johann (Hans) Edge

"Pain is the purifier"

— Percy Cerutty

Hans' favourite quote

Abstract

Repeated-sprint ability (RSA), power and strength are regarded as fundamental physical attributes for team-sport athletes. Investigations of concurrently training these fitness qualities, particularly in well-trained female team-sport athletes are scarce. The primary purpose of this thesis was the improvement of team-sport performance in female athletes through concurrent resistance (RT) and RSA training, concentrating on acute and chronic training structure. With recent interest in inflammatory cytokines in sports science, the cytokine response to concurrent exercise was also investigated with the aim of effectively measuring internal training load. The mechanism of action of interleukin (IL)-6, the most commonly researched cytokine in sports science, was also investigated.

Chapters 4 and 5 measured performance and magnitude of change in inflammatory cytokines and endocrine hormones to single mode or concurrent RT (6×6 sets of back squat exercise at 80 % 1-repetition maximum, sets separated by 3 min rest 2) and RSA (4×6 sets of 20 m maximal shuttle sprints, sets separated by 3 min rest) exercise of different intra-session exercise order (exercise modes separated by 15 min rest). It was found that exercise intensity and duration were important factors in the exercise induced cytokine and endocrine hormone response to exercise as significant alterations in these bio-markers were only seen following concurrent exercise (Chapter 5) and not during single-mode RT and RSA exercise (Chapter 4). There did not appear to be evidence of acute interference or residual fatigue present during the concurrent exercise protocol as intra-session exercise order did not affect maximal squat or sprint performance, or rating of perceived exertion in response to either mode of exercise. Exercise order showed minor differences in a small number of bio-markers.

Chapters 6 and 7 investigated the effects of 4 weeks of concurrent RT and RSA training, performed either on the same day (SDT: in a single session, 3 sessions per week) or alternating days (ADT: 6 sessions per week, each separated by 24h) on performance and systemic and skeletal muscle physiological responses to an acute concurrent RT/RSA protocol (protocol identical to RT:RSA exercise performed in Chapter 5). Both training structures were found to significantly increase RSA total time, sprinting, jumping and maximal strength performance. Interestingly, training structure (SDT or ADT) had no significant effect on the magnitude of these increases despite large differences in rest periods (15 minutes vs. 24 hours) between the exercise modes perhaps suggesting that the volume of the acute stimulus is less important than the total volume of training. The acute concurrent RT

and RSA protocol (both pre- and post-training) significantly altered systemic and local cytokines, hormones and signalling proteins within 180 minutes of the recovery period. However, training appeared to have no effect on the majority of these responses with only serum cortisol and plasma glucose concentrations significantly lowered by training. The failure of the inflammatory response to attenuate with training suggests that current understanding of cytokines and their role in the immediate post-exercise period is unclear and thereby reduces their effectiveness to provide information about training load.

IL-6 is the dominant cytokine responding prior to other anti-inflammatory cytokines in response to exercise and is also thought to play a role in exercise metabolism via the regulation of glucose output from the liver. In Chapter 8 a new method for measuring the role of IL-6 on hepatic glucose output (HGO) was tested using exercised human plasma and an isolated rat liver model. Results from Chapter 8 provide information suggesting that IL-6 may not increase HGO at rest or during exercise and may even negatively regulate HGO, in contrast to current theory.

The concurrent RT and RSA protocol employed throughout this thesis was shown to be an effective training stimulus to produce large increases in performance variables in female team-sport athletes in a short training period irrespective of exercise order or training structure. Based on the results of these studies and until the physiological role of the inflammatory cytokines elevated by acute exercise but unaltered by training are more clearly understood, it is not yet worthwhile for sport science practitioners to invest in cytokine monitoring in team-sport athletes for the measurement of training load.

Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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List of Abbreviations

1-RM	1- Repetition maximum
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
ADT	Alternating day training group
AMP	Adenosine monophosphate
AMPK	5' adenosine monophosphate-activated protein kinase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUC	Area under the curve
AU	Arbitrary units
BSA	Bovine serum albumin
CaMK	Ca^{2+} /calmodulin-dependent protein kinase II
CHO	Carbohdyrate
CK	Creatine kinase
CMJ	counter movement jump (check this spelling)
COD	Change of direction
CSA	Cross sectional area
CV	Coefficient of variation
CXC-2	CXC receptor-2
CXCR2	C-X-C chemokine receptor type 2
DDT	Dithiolthreitol
DJ	Drop jump
EIF4E	Eukaryotic translation initiation factor 4E
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyography
ES	Effect size

EUR	Eccentric utilization ratio
FOXO	Forkhead box 03
FFA	Free fatty acid
GH	Growth hormone
GLP-130	Glycoprotein 130
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter 4
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GXT	Graded exercise test
HGO	Hepatic glucose output
HIT	High-intensity interval training (check if I hyphen)
HPA	Hypothalamic-pituitary-adrenal axis
HR	Heart Rate
ICC	Intra-class correlation coefficient
IGF-1	Insulin-like growth factor 1
IGFBP	Insulin-like growth factor-binding protein
INF-γ	Interferon gamma
IL	Interleukin
IL-6R	Interleukin-6 receptor
ISAK	International Society for the advancement of kinanthropometry
JAK	Janus Kinase
JUNB	Transcription factor jun-B
KO	Knock out
MAFbx	Muscle atrophy F-box protein
MCR	Metabolic clearance rate
MHC	Myosin heavy chain
MIP	Macrophage inflammatory protein
mTOR	Mammalian target of rapamycin
MurF-1	Muscle RING-finger protein-1
OC	Oral contraceptive/pill
p70S6K	p70S6 kinase
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PKB	Protein kinase B/Akt
RER	Respiratory exchange ratio
RFD	Rate of force development
rh	Recombinant human
RPE	Rate of perceived exertion
RSA	Repeated sprint ability
RT	Resistance training/exercise
S6	ribosomal protein S6

SD	Standard deviation
SDT	Same day training group
SJ	Static jump
SOCS	Suppressor of cytokine signalling
SRT	combined resistance and sprint group
STAT	Signal transducers and activators of transcription
TBST	Tris Base, sodium chloride, Tween 20
TNF-α	Tumor necrosis factor alpha
TSC1/2	Tuberous sclerosis 1/2
TT	Time trial
VEGF	Vascular endothelial growth factor
YYIRT	Yo Yo intermittent recovery test

Ethical Approval

Ethical approval for the research carried out in Chapters 4-7 for this thesis research was granted by the Auckland University of Technology Ethics Committee (AUTEC). Ethical approval for the research carried out in Chapter 8 for this thesis research was granted by the University of Auckland Human Participants Ethics Committee (UAHPEC) and the University of Auckland Animal Ethics Committee (UAAEC).

AUTEC references were:
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Chapter 8: Ethics code: NTX/10/12/123 (human)
Ethics code: R1042 (animal)

CHAPTER 1

Introduction

Introduction

Current time-motion analysis studies show that team-sports are multi-dimensional, requiring high physical, physiological and technical abilities to cope with the demands of the game [1–7]. Team-sport athletes are required to perform short (< 5 s), powerful anaerobic bouts of activity, such as technical accelerative, jumping and tackling movements [8], as well as bouts of repeated-sprint activity [3,6,7,9,10] interspersed with lower intensity activities such as walking, jogging and backwards running. It has been shown that better (elite vs sub-elite) team-sport athletes can be distinguished by the quantity of high-intensity and sprinting efforts during match-play [6], repeated-sprint ability (RSA) [11] and maximal squat performance [12,13], which has been shown to correspond with acceleration, sprint and jumping performance [14–16]. This suggests that RSA, power and strength can be regarded as fundamental attributes for team-sport athletes and the development of these qualities should be considered and well-advised through training design.

However, training these qualities as well as technical and tactical training can put a lot of pressure on available time. Concurrent training may be used to optimise time use and to also increase specificity of training, as strength, power and sprint movements are performed simultaneously within match-play. Consideration of the interference effects of performing two differing modes of exercise within close proximity in a training programme is required.

The combination of traditional endurance exercise with resistance (RT) exercise has been shown to have detrimental effects on strength [17–19] and power development [17, 19, 20] with both acute (peripheral fatigue) and chronic (interference of competing molecular and morphological adaptations) mechanisms postulated to be responsible [21]. On the other hand, concurrent training with variations of RT and sprint exercises in male team-sport athletes have been shown to result in advantageous improvements in performance qualities important to team-sport match-play [22–30]. This suggests that the closer the two modes of exercise lie on the exercise continuum (strength-endurance) and the more similar they are in the work:rest ratio within each exercise mode, the more compatible they should be in terms of the training induced adaptations and subsequent performance improvements. Though, additional studies performed with trained female team-sport athletes are required to determine the effectiveness of concurrent RSA and RT exercise for the improvement of team-sport performance in this population.

The above studies mainly concentrated on investigating improvement in laboratory-based team-sport-specific performance variables (previously shown to be related to match-play performance), yet the physiological responses to concurrent RSA and RT exercise have received little attention to date.

When optimising training for a specific population and particular performance benefits, not only is it important to quantify external training load (i.e., intensity, duration and load) it is also important to understand the physiological stress of the training regime and how an athlete may tolerate the session. It is also critical to structure the exercises in a way such as to optimise the acute physiological response, ensuring the greatest potential for long-term adaptation. Therefore, viable bio-markers that may provide greater insight into the internal physiological stress experienced during and after exercise are required as measures of internal training load.

Current literature provides evidence that exercise appears to be an effective stimulus for inflammatory cytokines, that are elevated during and following exercise in an intensity, duration and skeletal muscle mass dependent fashion [31–34]. Further, inflammatory cytokines have been shown to be elevated in the systemic circulation and skeletal muscle in response to metabolic [35–37], eccentric [34,34,38] and concentric contractile stress [39–42] and likely induce a cascade of events necessary for skeletal muscle repair and adaptation. Therefore, it has previously been suggested that changes in inflammatory cytokines and hormones of the growth hormone↔insulin-like growth factor-1 (GH↔IGF-1) axis may be used to gauge training load [43, 44]. Inflammatory cytokines may be particularly useful when attempting to quantify internal training load between divergent modes of exercise that are not easily compared with simpler measures such as heart rate and ratings of perceived exertion due to differences in fatigue mechanisms [45]. Measures of bio-markers may provide valuable information regarding exercise tolerance, and adaptive responses to training, that may help strength and conditioning and sport science practitioners structure and adjust training parameters for optimal improvements in physical performance. It is also likely that the immune/inflammatory system does not act in isolation and an exploratory analysis of the combined endocrine, metabolic and molecular responses will provide a greater understanding of the acute physiological stress and potentially a greater insight in to the physiological adaptation involved in the improvement of performance.

Relatively few reports have examined markers of training stress in female team-sport athletes in the pre-season training period. Specifically, the ease of measuring and monitoring inflammatory cytokines for quantification of internal training load in this population has yet to be investigated. Additional studies are therefore required to assess the potential for using inflammatory cytokines as measures of acute internal training load and short-term adaptive responses in trained female team-sport athletes.

Purpose statement and significance of study

The purpose of this thesis was to (1) establish the efficacy of concurrent RSA and lower-body RT exercise training on the development of team-sport specific performance variables in trained female team-sport athletes. (2) Investigate the effectiveness of using exercise induced alterations in inflammatory cytokines as a measure of internal training load, and their relationship with physical improvements in performance. Within the context of this purpose statement and adding to the limited research in team-sport specific concurrent training this thesis aimed to:

- determine the systemic inflammatory cytokine and endocrine response to acute single mode RSA and lower-body RT exercise in trained female team-sport athletes.
- determine the effect of exercise order within an acute concurrent RSA and lower-body RT exercise session on performance in trained female team-sport athletes.
- determine the effect of exercise order within an acute concurrent RSA and lower-body RT exercise session on systemic inflammatory cytokine, endocrine, and white blood cell responses in trained female team-sport athletes.
- determine the effectiveness of 4 weeks of same day or alternating day concurrent RSA and lower-body RT training in improving team-sport performance variables in trained female team-sport athletes.
- determine the systemic and local skeletal muscle inflammatory cytokine responses to acute concurrent RSA and lower-body RT exercise both before and following 4 weeks of concurrent training in female team-sport athletes.
- determine the systemic endocrine and metabolic as well as the molecular signalling responses to acute concurrent RSA and lower-body RT exercise both before and following 4 weeks of concurrent training in female team-sport athletes.

The series of studies contained within this thesis will enhance current understanding of the compatibility of RSA and lower-body RT training specifically in female team-sport athletes from both an acute and chronic perspective. The information provided by Chapters 4-7 may help to inform training programme design to maximise adaptations specific to both RSA efforts and powerful movements required of team-sport athletes. This may be accomplished by understanding the effect of intra-session exercise order on subsequent second mode performance, and understanding the role of intra-exercise recovery period on acute and chronic performance adaptation. Further, the understanding of inflammatory cytokines as markers of exercise intensity, metabolic stress, tolerance of exercise and adaptation from both an acute and chronic perspective will be enhanced.

Thesis organisation

The thesis comprises 9 chapters. Each of the experimental Chapters 4-8 are written as stand-alone chapters that incorporate standard paper format (abstract, introduction, methodology, results, discussion) and thus are specific to the aims of that chapter. These experimental chapters are integrated between Chapters 1 and 9 which introduce and discuss the body of work as a whole. Chapter 1 introduces the thesis topic, outlines the purpose and individual aims of the research and outlines the structure and flow of the thesis (Figure 1.1).

The thesis comprises two literature reviews (Chapters 2 and 3) due to the complexity of concurrent exercise training and exercise induced inflammatory cytokine responses, these topics are discussed in their own right in separate chapters. Specifically, Chapter 2 briefly reviews literature on traditional concurrent training (endurance and RT) before discussing the physical and physiological determinants of team-sport performance. Concurrent RSA and RT exercise training with particular reference to team-sport athletes is then reviewed and discussed in detail. Chapter 3 reviews the current literature on systemic and local skeletal muscle cytokine responses to acute and chronic exercise within healthy adult populations.

Chapter 4 was designed as an important precursor to the proceeding experimental chapters (Chapters 5-7) and investigates the physiological, inflammatory cytokine and endocrine hormone responses to single-mode RSA and RT exercise. In order to determine the possible interference or additive effects within a concurrent exercise regime it was important to understand the responses to individual stimuli first (RSA or RT exercise). Also, in Chapter 4, a time course of both cytokine and endocrine responses was conducted in order to determine the optimal timing of blood sampling in proceeding chapters (Chapters 5 and 7).

Chapter 5 explored the role of the intra-session exercise order on the acute inflammatory cytokine and endocrine response and determined the role of the first mode of exercise on the ability to maximally perform the second mode of exercise. The following two chapters employed a logical progression from Chapter 5, and using the information gained about the role of intra-session exercise order within the specific concurrent RSA and RT exercise regime, a 4 week training intervention was designed and implemented. Chapter 6 determined the effectiveness of multiple acute concurrent RT and RSA training sessions, of two differing structures, on team-sport performance variables in trained female team-sport athletes.

Chapter 7 explored the role of 4 weeks of concurrent training on the acute exercise induced systemic and local cytokine, endocrine and metabolic hormone responses and molecular signalling cascade to concurrent RT and RSA exercise in a trained female population in order to understand the adaptive responses that

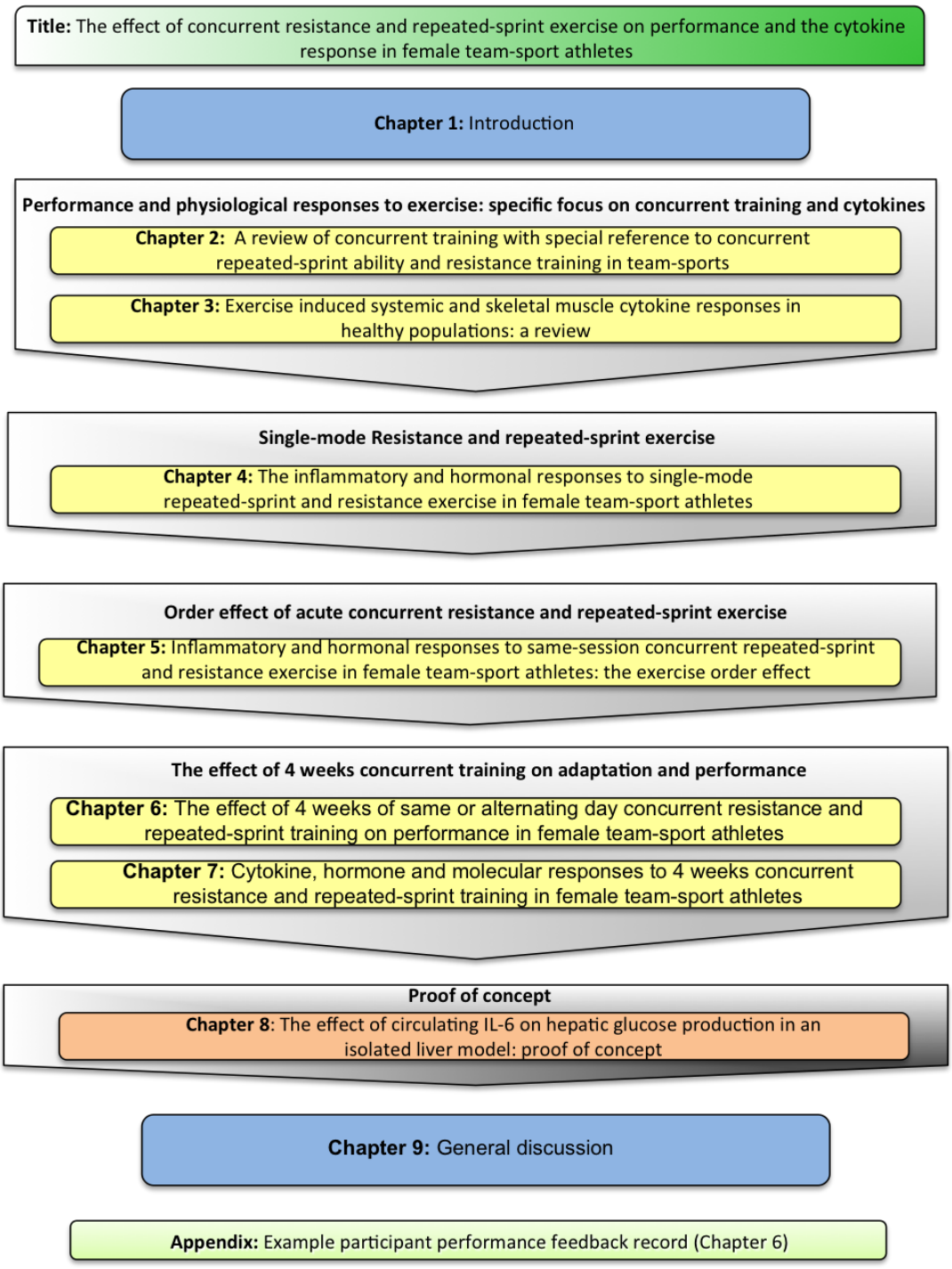


Figure 1.1: Overview of the thesis structure.

support performance improvement.

An additional chapter (Chapter 8) was added to the thesis to test current theory that IL-6 can influence carbohydrate metabolism through increasing hepatic glucose output (HGO) during exercise. A brief prelude to this chapter explains how the findings of the preceding chapters led to the addition of this chapter. A proof of concept study was designed to test the direct role of IL-6 on HGO at rest and during exercise through a novel isolated liver method. The final Chapter 9 summarises and

discusses the results of the thesis as a whole before providing concluding remarks on the efficacy of concurrent RT and RSA training in improving team-sport performance and adaptive responses in a trained female team-sport population. The chapter also discusses areas for future research.

Due to the structure of the thesis there will be repetition of methodology and terminology within the experimental chapters, however minor adaptations to methodology will occur in successive chapters. For consistency and readability there is a single reference list of citations included at the end of the thesis.

CHAPTER 2

A review of concurrent training with special reference to concurrent repeated-sprint ability and resistance training in team-sports

Abstract

As part of tactical matchplay, team-sport athletes are regularly required to perform repeated powerful movements including but not limited to jumping, tackling, changing direction and accelerating. A large aerobic capacity is required to facilitate recovery from these high-intensity efforts as well as sustain low-intensity periods of match-play. Further, both repeated-sprint ability (RSA) and maximal power have been deemed important determinants of team-sport performance and therefore concurrent training is required to be undertaken in team-sport athletes. Traditional concurrent endurance and resistance training has been shown to result in an attenuation of strength gains, and in particular explosive power when endurance and resistance exercise are combined. However the majority of research suggests that endurance performance is generally unaffected. Adaptive responses, including endocrine, molecular and neuromuscular differ depending on the configuration of the concurrent exercise, and therefore may determine the compatibility of adaptive response. As RSA and resistance exercise can be similar in force production and work:recovery ratio, the purpose of this review is to analyse the current concurrent RSA and resistance training literature in order to determine the compatibility of the training adaptation in order to optimise increases in RSA and explosive performance in team-sport athletes. The traditional models of concurrent endurance and resistance exercise will be reviewed first to provide a platform in which to discuss a role for concurrent RSA and resistance exercise.

Introduction

Team-sport athletes represent the best example that concurrent training can work to achieve adaptations that are both specific to aerobic fitness and dynamic strength. Concurrent training is the combination of two deviating modes of exercise incorporated into a single periodised training programme. Classically, studies of concurrent training have aimed to determine the interactive effect of the two traditional forms of exercise, aerobic and resistance training [17–20, 46, 47]. Endurance exercise training is traditionally of several minutes to hours in duration and is associated with improved capacity to sustain low resistance, high-intensity contractions. Strength or power training typically comprises high force, lower volume contractions and is relatively short in duration and is associated with increases in strength and power. Given that such a large number of sports such as soccer, rugby, hockey and netball require combinations of aerobic, anaerobic, speed, strength and power parameters for optimal performance, research has attempted to determine the physiological and performance cost of completing strength and endurance exercise within the same training program [12, 17, 19, 48–50].

Adaptations to traditional concurrent training

'Traditional' concurrent training investigations that have compared concurrent resistance and endurance exercise with resistance only training have shown that concurrent training appears to result in significant decrements in strength gains [17–19], muscular power [17,19,46], and muscle hypertrophy [18,19,46]. However some investigations found small or no decrements in gains from strength training in combination with endurance training [20,46,47]. While occasionally decrements in endurance performance have been reported with concurrent endurance and resistance training, the majority of studies provide evidence that endurance development most commonly measured by estimates of maximal aerobic power are not impaired within a concurrent training regime [18–20, 51]. Therefore, it appears the most notable considerations with concurrent training is how the endurance exercise interferes with the neuromuscular system causing an acute inability to generate maximal force and chronic inability to gain strength.

The interference phenomenon, first described by Hickson [18] describes the potential for two modes of exercise to inhibit the normal and optimal response achieved when the modes of exercise are performed in isolation. A number of theories have been postulated to try to explain or understand the interference often reported with concurrent resistance and endurance training. The theories can be grouped in to two major classifications 1) the acute hypothesis and 2) the chronic hypothesis [21].

Acute hypothesis

The mechanisms at play in the suppression of the optimal resistance exercise response have been difficult to elucidate due to a number of inconsistencies in study design, including but not limited to: participant training history, duration, mode and intensity of exercise [21]. However, the acute divergence effect of concurrent endurance and resistance training may be caused by peripheral fatigue factors that are residual of the endurance training [52]. Craig et al. [52], proposed this theory suggesting that residual fatigue (glycogen depletion, muscle damage and inflammation) may reduce the ability to produce tension in the skeletal muscle, reducing the stimulus effect on muscle building pathways culminating in diminished strength development over time. In support, Sale et al. [51] observed greater increases in strength when concurrent endurance and resistance training were performed on alternating days suggesting a greater recovery likely reduces residual fatigue effects and allows the second mode of exercise to be performed maximally.

Further, performing exercise in a lowered glycogen state may enhance the metabolic stress of the second exercise mode. During endurance exercise this has resulted in net protein breakdown [53] and during resistance exercise this has resulted in impairment of anabolic signalling pathways [54]. However, Churchley et al. [55] and Camera et al. [56] both utilised one-legged models to reduce glycogen content in one leg (cycling exercise to fatigue) which was followed the next day by unilateral resistance exercise (80 % 1-RM) in both legs (equating to low and normal muscle glycogen content). They did not observe any detrimental (or enhancement) effects on genes or proteins regulating hypertrophy nor rate of myofibrillar protein synthesis in the low glycogen leg compared with the control leg. Thus, suggesting the anabolic response in the immediate recovery period during concurrent exercise may not be compromised by low muscle glycogen availability.

The intra-session exercise order may therefore be important to consider during same-session concurrent training for chronic performance adaptation. Interestingly, even though it is likely that the recovery time between the successive modes of exercise would be expected to influence the adaptive responses to concurrent training, no studies have specifically investigated the timing of recovery within such a structure.

Chronic hypothesis

The chronic hypothesis relates to the chronic incompatibility of the skeletal muscle to adapt to two distinctive modes of exercise at the same time [21]. The effects of resistance training on alterations in muscle size, substrate metabolism and contractile efficiency (recruitment, velocity, force) are at often-times markedly variant

from those alterations caused during adaptation to endurance exercise [57]. A conflict therefore exits within skeletal muscle when adapting to concurrent training and therefore may be unable to adapt optimally to both stimuli resulting in attenuated adaptive responses when compared with adaptations to isolated single mode exercise.

Molecular adaptation

The two distinctive skeletal muscle adaptations of endurance (low-force, high repetition) versus resistance training (high-force production, low repetition) logically pose the possibility of cross-talk inhibition between the distinctive molecular adaptive pathways of endurance and resistance exercise [58].

Heavy contractile loads lifted during resistance exercise are associated with overload-induced skeletal muscle hypertrophy via the facilitation of a specific cascade of intracellular events associated with the Akt-protein kinase B (PKB)/mammalian target of rapamycin (mTOR) molecular pathway [59, 60]. mTORC1 is known to phosphorylate and thus activate eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and p70S6 kinase (p70S6K), both proteins involved in the stimulation of translation initiation [59]. While low-force, high repetition contractions performed during traditional endurance exercise are associated with increases in mitochondrial content via activation of the 5' adenosine monophosphate-activated protein kinase (AMPK)-peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) molecular pathway [59, 60]. However, relatively recently AMPK has been shown to be an antagonistic factor with the potential to inhibit anabolic processes that demand adenosine triphosphate (ATP) breakdown [61]. AMPK has been shown to down-regulate the activity of mTORC1 by direct phosphorylation of tuberous sclerosis proteins 1 and 2 (TSC1/2) [61,62]. Further, AMPK has also been shown to promote forkhead-box (FoxO)-dependent transcription of muscle-specific ubiquitin ligases MaFbx (muscle atrophy f-box) and MuRF-1 (muscle ring-finger 1) [63, 64] which contribute to a catabolic environment by up-regulating protein degradation. This effect can be mediated by the activation of the Akt-signalling pathway, which can act to down-regulate the transcription of these catabolic mediators [65, 66].

It is thought that the metabolic cross-talk between the Akt/mTOR pathway and the AMPK-PGC-1 alpha pathway is in part responsible for the impairments in strength development during concurrent endurance and resistance training (refer to Figure 2.1).

Further, while it has long been thought that endurance training does not result in a positive protein balance i.e. net protein synthesis; recent reports have shown

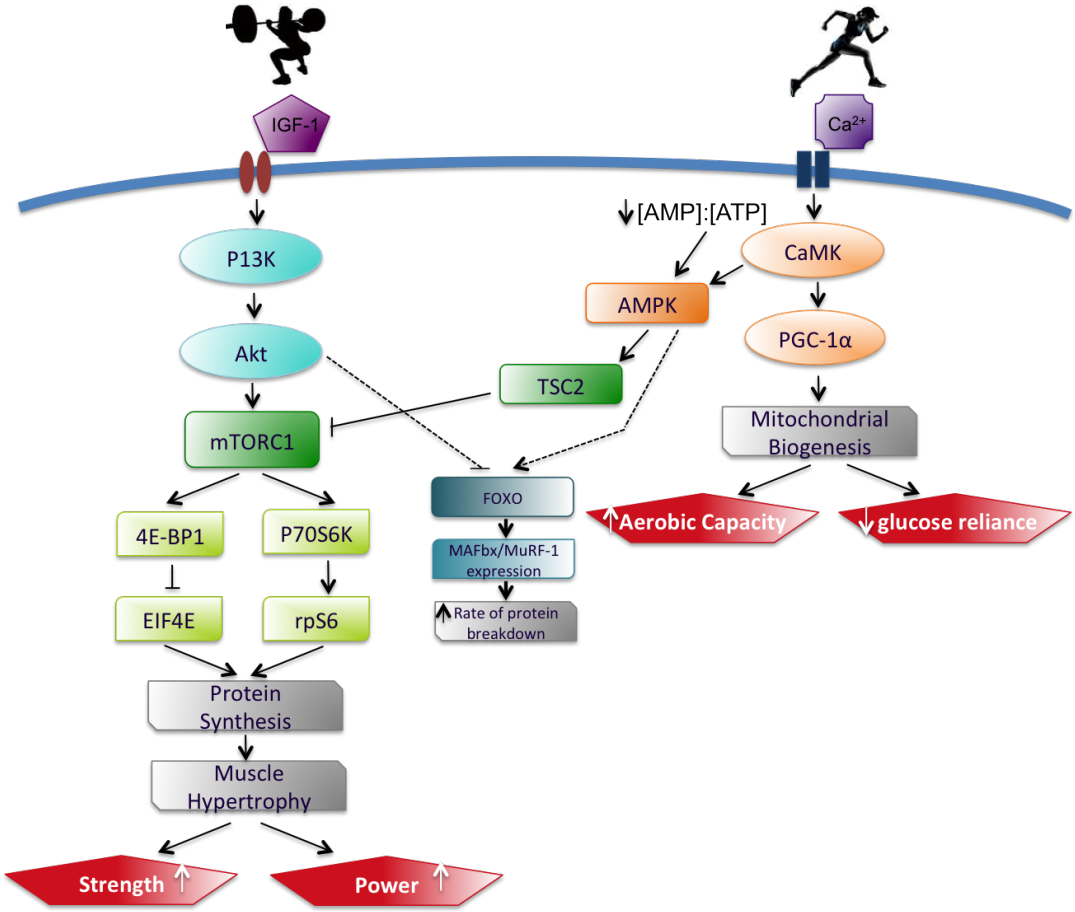


Figure 2.1: Simplified diagram of possible molecular interference with traditional concurrent resistance and endurance exercise. IGF-1 = Insulin like growth factor 1. Akt = Protein Kinase B. mTORC1 = Mammalian target of rapamycin complex 1. 4E-BP1 = Eukaryotic translation initiation factor 4E-binding protein 1. EIF4E = Eukaryotic translation initiation factor 4E. p70S6K = p70S6 kinase. TSC2 = tuberous sclerosis 2. AMPK = 5' adenosine monophosphate-activated protein kinase. CaMK = Ca^{2+} /calmodulin-dependent protein kinase II. PGC-1α = Peroxisome proliferator-activated receptor gamma co-activator 1-alpha. AMP = Adenosine monophosphate. ATP = Adenosine triphosphate. FOXO = Forkhead box O3. MAFbx =Muscle atrophy F-box protein. MuRF-1 = muscle RING-finger protein-1

that endurance exercise also results in elevated protein synthesis and activation of the insulin-like growth factor 1 (IGF-1) signalling pathway [67, 68]. However, there may be a difference in the type of proteins developed following endurance as opposed to resistance exercise, with greater mitochondrial protein synthesis present following endurance exercise and myofibrillar protein synthesis following resistance exercise [69]. Therefore, despite similar increases in protein synthesis, the divergent contraction modes with endurance versus resistance exercise are likely to induce different functional protein accretion and resultant exercise-induced adaptation. In support of this, Donges et al. [70] determined myofibrillar and mitochondrial protein fractional synthesis rates (FSR) after isolated bouts of resistance exercise (RT = 8 x 8 leg extension at 80 % 1-RM), aerobic exercise (AE = 40 min cycling at 55 % peak

aerobic power output) and concurrent exercise (CE = 50 % of each of leg extension and cycling exercise) in sedentary middle-aged men and observed equivalent rates of mitochondrial FSR between RT, AE and CE exercise, as well as similar increases in myofibrillar FSR between RT and CE despite combined exercise consisting of only 50 % the volume of isolated AE or RT exercise. While these results were found in the absence of an interference effect, it is currently unknown how these responses translate into a chronic training scenario and whether the development of functional strength and aerobic capacity would be similar between isolated and combined exercise.

Some acute studies have provided evidence of molecular interference or incompatibility when completing concurrent exercise within the same session and of different intra-session exercise orders [71–73]. However, Wang et al. [74] reported positive molecular responses to combined endurance and resistance exercise when successive bouts of endurance (1 h cycling at 65 % $\dot{V}O_2$peak) and resistance exercise (6 sets of leg press at 70-80 % 1-repetition maximum (1-RM)) amplified the adaptive signalling response of mitochondrial biogenesis when compared to endurance only exercise. This suggested that concurrent endurance and resistance exercise may be beneficial for increases in maximal aerobic capacity. However, the genes related to muscle building pathways were not measured in this study. Also, post-exercise signalling and/or protein synthesis are not necessarily correlated with resistance training induced hypertrophy [75, 76]. Thus, making predictions of chronic molecular adaptation and/or interference and possible associated performance adaptation from acute signalling studies difficult and may provide limited information for the strength and conditioning practitioner.

The chronic molecular responses to concurrent training are yet to be widely investigated. De Souza et al. [77] reported differences in molecular adaptations pre to post 8 week of strength training (increased AktSer473 phosphorylation), interval training (increased AMPK phosphorylation), or combined strength and interval training (increased total p70S6k protein content) but concurrent interval and resistance training did not blunt muscle strength or hypertrophy increments when compared to strength training alone.

Thus it appears the molecular mechanisms governing adaptation to concurrent training are far from understood and additional research is required to determine the importance of these responses within a concurrent training regime and how they may translate to performance [for an in depth review of the molecular bases of concurrent training see [78]].

Fibre type/hypertrophy

Different contractile stimuli have been shown to elicit differences in muscle-fibre composition and myosin heavy chain (MHC) isoforms. Endurance exercise has shown a switch from fast to slow MHC isoforms [79, 80] whereas resistance exercise has been shown to reduce type IIx MHC isoform and increase the proportion of type IIa fibres [81]. Changes in muscle-fibre composition can therefore alter the metabolic and contractile properties of the skeletal muscle [82] and to understand the effect of two differing contractile activities on fibre type transformations, fibre type adaptations have been investigated with concurrent training. However, inconsistencies in patterns of fibre type hypertrophy have been reported. An impairment of type 1 fibre hypertrophy was recorded by Kraemer et al. [19] in response to concurrent endurance and strength training whereas Nelsen et al. [83] reported type I, type IIa and IIx hypertrophy in response to concurrent endurance and strength training but only type IIx hypertrophy in the strength only group. In contrast, Hakkinen et al. [17] reported no difference between strength and strength and endurance groups in fibre cross sectional area (CSA) of type I, type IIa and IIx fibres. Similarly McCarthy et al. [46] reported similar increases in type II myofibre areas between strength and strength and cycling groups. Therefore, research to date suggests that concurrent training may modestly alter the pattern of transition of muscle fibre type and composition that occurs when resistance training is done in isolation. There is a need for further research with more sensitive analytical techniques in a broader range of concurrent exercise regimes to determine possible interference effects on fibre type transformations. Importantly how these transformations translate to whole muscle performance requires further investigation.

Neuromuscular adaptations

In addition to gains in strength from muscle hypertrophy, development of the neuromuscular system can be a significant determinant of contractile efficiency in skeletal muscle [84]. The force produced by the whole muscle is a product of the efficiency of recruitment and number of motor units activated and the rate at which the motor unit can produce an action potential [84]. Neuromuscular adaptations seem particularly important during the beginning phases of strength training [85,86] and in the development of maximal [87,88] and explosive strength [89–92].

The effect of concurrent training on the neuromuscular improvements with training are ambiguous. In comparison to endurance training alone, 8 weeks of concurrent endurance and explosive strength training improved force time characteristics and increased rapid neural activation of the quadriceps in runners with no decreases in aerobic capacity despite a 19 % lower endurance training volume [93].

However, in trained endurance cyclists, 12 weeks of concurrent cycle endurance and strength training attenuated 1-RM leg strength, squat jump (SJ) performance and rate of force development (RFD) compared with strength training in isolation [94]. While Hakkinen et al. [17] reported similar increases in maximum strength gains, maximum electromyography (EMG) activity and enlargement of CSA of whole muscle and individual fibres in a strength training only and concurrent endurance and strength training group. However, the concurrent training group attenuated the development of explosive strength by limiting rapid neural activation. Similar to the role of concurrent training on fibre type transformations, the effect of concurrent training on neuromuscular adaptation in comparison to resistance only training is unclear. It does appear however, that endurance training performed concurrently with resistance training, may be likely to interfere with explosive strength or power development compared with maximal strength, possibly due to interference in the development of voluntary rapid neural activation. Further research may help to determine the effects of endurance exercise on the force-velocity adaptations to resistance exercise in the developing muscles.

Sequencing of endurance and resistance exercise

While a number of different sequencing and ordering effects of endurance and resistance training have been implemented in prior research, including both same and separate day concurrent training, the specific effects of same-session scheduling of endurance and resistance exercise has gained less attention [50, 95–97]. The order effect of concurrent exercise has shown mixed results with Cadore et al. [95] demonstrating that the strength-endurance concurrent order resulted in greater lower-body strength gains as well as greater changes in the neuromuscular economy of vastus lateralis in older individuals compared with the opposite order. In contrast, in male sport students Chtara et al. [96] found that same session endurance-strength order produced greater improvement in 4 km running time trial performance and aerobic capacity (8.6 %) than the opposite order (4.7 %) following 4 months training of intermittent endurance exercise (5 × 100 % of v$\dot{V}O_2$max: active recovery at 60 % of v$\dot{V}O_2$max) and resistance exercise (explosive lower body movements, including jumping and bounds in a circuit for 30 mins). However, in similar participants Chtara et al. [97] found the order of combined circuit resistance training and high-intensity endurance intervals separated by 15 mins (same exercise training as Chtara et al. [96]) did not influence the adaptive response of muscular strength or explosive power [97] following 12 weeks of training. Similarly, the sequence of resistance (45 mins of 5-6 lower body exercises designed to increase strength) and rowing endurance exercise (45 mins at 70 % $\dot{V}O_2$max) performed with no rest did not influence strength training adaptations in physically active women following 11 weeks of train-

ing but may have limited aerobic adaptations in the resistance:row order [50]. These studies suggest that the order of same-session endurance and resistance exercise does not effect strength development, but that development of aerobic capacity may be reduced if endurance exercise is preceded by strength exercise during training.

Acute studies investigating hormonal responses to combined endurance and resistance exercise suggest the testosterone response (in males) is optimised in the endurance-strength order [98, 99]. Hormonal changes in response to each mode of exercise during concurrent training may ascertain how compatible the adaptations of each mode of exercise are when performed in close proximity. In particular, high intensity endurance exercise that relies on glycogen or results in accumulation of blood lactate has been attributed to higher acute cortisol responses [100–102] which may influence strength gains by catabolically causing protein degradation, shifting the paradigm from increased net protein synthesis following strength training. Reductions in hypertrophic adaptations following strength training may limit gains in strength [19]. However Schuman et al. [103] report differences in hormonal responses seen between exercise orders during week 1 of concurrent endurance and resistance exercise are diminished following 24 weeks of training (strength loading = 2-5 $\times$ 3-10 repetitions at 40-95 % 1-RM of dynamic leg press, leg extension and flexion; endurance exercise = 30-50 min of cycling ergometer steady-state and interval sessions above and below aerobic threshold) and are not associated with strength development.

The findings of a disconnect between acute post exercise hormonal responses and chronic strength development in the Schuman et al. [103] study are similarly supported by West et al. [104] who have recently demonstrated that acute post-resistance increases in proposed anabolic hormones testosterone, GH and IGF-1 were not required to stimulate skeletal muscle hypertrophy or increase strength in the elbow flexors during chronic resistance training (15 weeks). West and Phillips [105] observed a similar lack of correlation between acute exercise-induced systemic hormonal responses and leg-press strength following 12 weeks of resistance training. Importantly, these studies have utilised untrained male participants and it currently remains unclear if a similar lack of association between acute exercise induced anabolic hormones and skeletal muscle anabolism and strength gains exist in trained and/or female populations. Further, it has been previously argued that the supplementation with whey protein prior to and following resistance exercise in the West et al. [104, 105] studies may have been a confounding factor that may have masked the potential anabolic effects of the acute exercise induced hormone elevations [106]. Continued debate surrounds the role of exercise induced alterations in anabolic hormones and their relationship with skeletal muscle anabolism and strength [for in-depth reviews on the hormone hypothesis see [107, 108]].

It appears that intra-session exercise sequence may have an effect on the performance adaptations to concurrent endurance and resistance exercise with detriments more likely in the mode of exercise performed second, and detriments may or may not derive from hormonal variations. The rest interval allowed for between exercise modes may also influence acute adaptive interference but more work is needed in this area to confirm the most appropriate rest interval which is likely to be influenced by the specifics (type, intensity, duration) of both the endurance and resistance protocols.

Summary

The effectiveness of 'traditional' concurrent training in achieving performance benefits at both ends of the exercise continuum is likely to be a combined result of the structure of the concurrent training (same session or separate session), intensity and duration of the aerobic exercise, the percentage of maximal strength used and muscle mass involved during resistance exercise. Chronically, it may be that skeletal muscle simply cannot cope simultaneously with metabolic adaptation to both modes of exercise.

Physiological and performance requirements of field based team-sport athletes

Introduction

Team-sports typically involve coordinated and facilitated movements between a number of players in order to achieve the objectives of the game directed towards achieving a win. Due to the uniqueness of the demands of team-sport players a number of time-motion analysis studies have attempted to clarify specific movement patterns and physiological demands during match-play [1–7]. By understanding the physiological and performance requirements of team-sport players the strength and conditioning practitioner may be better able to structure training in order to improve specific performance variables that may ultimately improve match performance.

Physiological demands

Soccer players have recorded high total distances covered during match-play compared with other team-sport athletes with between 10-13 km covered during the course of a match [109, 110]. Soccer also has the longest match duration of 90 min in comparison to 80 min for rugby union and league, and 60 min for netball and basketball which may account for some of the difference in match distances covered.

Competitive rugby union and league players also cover large running distances during match-play with an excess of 6 km [9] and 9 km [111] recorded respectively, while hockey players also travel in excess of 6 km [7]. Due to the smaller size of the playing area, shorter match time and technical differences in game-play, netball and basketball players cover the least distance during match-play (∼4-7 km) [3].

During team based sports, a large majority of the game is spent in low intensity activity, with up to 65-97 % of total distance covered in low-intensity to moderate intensity forward walking and jogging movements [6, 7, 9]. This is most likely a reflection of the number of high-intensity maximal effort sprints and high-intensity movement activities including rapid changes in direction and jumping that take place at intermittent intervals throughout game-play [3, 5, 112] that require large amounts of recovery. Due to the portion of match-play that is spent completing high-intensity activities such as maximal sprints, tackles and jumps, large anaerobic energy provisions are required to support these intermittent high-intensity movements [8]. In support of this, moderate to large blood (7-10 mmol.L^{-1}) and muscle (16.9 ± 2.3 mmol.kg^{-1}d.w.) lactate levels have been recorded after intensive periods of team-sport play [4, 113–115] and are indicative of periods of high anaerobic energy turnover. Maximal sprint efforts occur up to ∼29, ∼33, and ∼39 times during match-play in rugby union, hockey, and soccer respectively [3, 6, 7, 9, 10], with typical distances of 14-20 m recorded during rugby, hockey and soccer for average sprint distance, of durations between 1-4 s [9, 116, 117]. Further, during elite male field hockey, repeated-sprint bouts (<3 consecutive sprints) were recorded on average 17 times per game, with an average recovery between consecutive sprints of 21 s [7]. Recovery from these maximal efforts are largely catered for by the aerobic energy system which is indicated by match intensities of ∼85-90 % maximal heart rate (HR) and ∼70-80 % of maximal aerobic capacity [1,7,110,118]. Therefore, high $\dot{V}O_2$max scores of between 50-70 ml.kg^{-1}.min^{-1} are commonly reported in elite team-sport athletes [5, 7, 119]. The repeated-sprint efforts typical of team-sport athletes are considered a specific fitness quality; repeated-sprint ability (RSA). Girard et al. [120] describe this fitness quality as the ability to perform repeated short sprints (< 10 s) interspersed with brief rest intervals (< 60 s).

Differences in a number of physiological characteristics are observed based on positional requirements and training status of the athletes. Differences in time spent completing high-intensity running (28 %) and sprinting (58 %) differ considerably between top-class and moderate professional soccer players [6] with top class players also performing better (∼11 %) on the Yo-Yo intermittent recovery test (YYIRT) [6]. Importantly, it has been found that mean time recorded during a field based RSA test (6 × 40 m shuttle sprints separated by 20 s recovery) was significantly positively correlated with both total distance covered during very high-intensity running and

sprinting during a professional soccer match [11]. Therefore, it seems likely that RSA is an important determinant of team-sport performance, and understanding how to improve this type of fitness quality alongside improving power and strength measures is important.

Strength and power

Strength and power are important co-determinants of team-sport performance, with combinations of strength and power required for sprinting, tackling, jumping and change of direction movements [121,122]. Maximal strength has a strong association with team-sport specific explosive power requirements, with a strong relationship between half squat 1-RM and acceleration and jumping ability [14–16, 123]. Maximal half squat performance also differs significantly between better performing and lower performing premiership league soccer players [12] and maximal half squat performance correlates strongly with sprint performance and jump height in elite soccer players [16]. Greater mean 1-RM squat (17 %) and SJ maximal power (11.5 %) are reported in elite compared with state league rugby players from the same club [13]. Further, in elite rugby players sprint momentum measured as the product of body mass and average velocity over 10 m has been shown to be an important physical characteristic which would provide particular advantage during flying tackles or collisions [13] producing greater impact forces on opposing players. Rugby players may also sustain between 800 and 1300 physical impacts during a single match performance [2] requiring the ability to forcefully counteract these impact forces to maintain balance and possession of the ball.

Lorenz et al. [124] compared data of elite and non-elite athletes in anaerobic field and court sports to try to determine predictors of elite performance and concluded that maximal power output, as used in accelerating and jumping, is the most prognostic measure of elite field/court sport performance. Interestingly, Wilson et al. [125] concluded following a meta-analysis of concurrent aerobic and resistance studies, that overall power (mean effect size (ES) for power development for strength training only was 0.91; for endurance training, it was 0.11; and for concurrent training, it was 0.55) may be more susceptible than strength (strength only 1.76; endurance only, 0.78; concurrent training, 1.44) and muscle hypertrophy (strength only 1.23; endurance only, it was 0.27; concurrent training, 0.85) to decrements when performed alongside aerobic exercise. This suggests that impairments of adaptations involved in the development of contractile velocity or RFD may occur when aerobic type exercise is performed concurrently with resistance exercise.

Concurrent repeated-sprint ability and resistance training for team-sport athletes: a review of current literature

As RSA and strength and power are important determinants of team-sport performance, recent research has attempted to determine the practicality and effectiveness of concurrently training these two important fitness qualities [22–30,72,126]. Of the 11 investigations that have to date studied concurrent RSA and resistance/strength training of variable methodological designs, eight were carried out in competitive team-sport athletes [22–28, 30, 126] (including 4 in soccer players, 2 in basketball players and 1 in rugby players). Only one study has investigated concurrent RSA and resistance exercise in females [126]. All but one [22] of the investigations in team-sport athletes were carried out during the in-season phase and were therefore supplementary to regular team-sport skill and tactical trainings. Ten of the studies included a training period in the intervention of between 6-20 week duration and were focused on performance outcomes. Only one study addressed the acute molecular responses to concurrent RSA and resistance training, and only one study addressed the effect of intra-sequence ordering. Each of these studies are now reviewed in detail.

Tsimahidis et al. [23] studied 26 healthy competitive junior basketball players ($\sim$ 18 y) who completed 10 weeks of high-intensity resistance training (combined training group CTP) consisting of half squat exercise (5 weeks = 5 $\times$ 8 RM, 5 weeks = 5 $\times$ 5 RM) with each squat set followed immediately by a maximal 30 m sprint or completed only the regular technical or tactical team basketball sessions (control group, CON). Large improvements were seen in maximal back squat following 10 weeks of training ($\sim$30 %) in the CTP group, with no increases seen in the CON group. Maximal 0-10 and 0-30 m, SJ, countermovement jump (CMJ) and drop jump (DJ) performances were all significantly increased following 5 and 10 weeks of training in the CTP group. This study consisted of only 5 maximal 30 m sprints per training session, but in combination with the resistance training, players were able to greatly improve the acceleration phase, and maximal velocity phase of a 30 m sprint and improve jumping performance, all of which are important physical characteristics of basketball. However, as the sprints weren't 'repeated' (i.e. sprints conducted multiple times within a short period), it is unclear whether RSA ability would have been improved with this protocol. Also, as there was no resistance only training group, it is also difficult to determine the contribution of the 5 single sprints performed per session to overall performance improvements. As half squat strength is correlated with sprinting performance in team-sport athletes [16] and due to the ratio of squats to sprints in the training protocol, increases in maximal strength likely made the greatest contribution to improved linear sprint performance.

Similar improvements in maximal sprinting and jumping ability were recorded

by Alonso et al. [24] in another sub-population of competitive basketball players (∼18 y) with little to no previous strength training experience following 6 week combination of sprint exercise, resistance exercise and plyometrics. Weeks 1-3 consisted of RSA (2-7 × 20-40 m sprints with 20 s rest between sprints) and half squat (4-5 sets × 6-10 reps with 3 min rest with the concentric phase completed as explosively as possible) exercise. Weeks 4-6 consisted of half squat and plyometric jumping exercises (2 × 6 reps of: stiffness jumps, jump squats, hurdle jumps, drop jumps). Training was undertaken alongside 2 × regular basketball trainings and 1 game per week (n=7). A control group (n=6) completed the same volume of training by including extra team basketball trainings. SJ and CMJ were significantly increased in the experimental group by ∼3 and 4 cm respectively, while small decrements were recorded in the control group. Weighted jump squat (with a load equal to 200 % body weight) increased significantly by ∼6 watts per kg, and agility and 40-m shuttle were improved in the experimental group only. YYIRT also increased by 463 m in the experimental group. Regrettably, RSA was not measured in this study which is unfortunate as unlike with the previous study, the specificity of the RSA training employed would have increased the likelihood of improving components of RSA [127].

Increases in jump and sprint performance were also been seen by Marques et al. [26] in young soccer players (∼13.5 y) who utilised concurrent plyometric jumping exercises (2 legged jumps, hops) and RSA efforts that (from standing 6 × 20 m, flying start 5 × 30 m) focused on ground contact and increasing lower limb explosive power. Plyometric jumping and RSA were completed for 20 min twice a week for 6 weeks alongside four regular team-trainings (n=26). A control group completed regular team-trainings only (n=26). CMJ was increased by 7.7 % in the experimental group and remained unchanged -(1.1 %) in the control group. Further, the experimental group improved 0-30 m and 15-30 m sprint times but did not improve the acceleratory phase (0-15 m). Plyometric exercise may therefore be a suitable substitute for heavy squat exercise in young developing athletes. Again, like the Alonso et al. [24] study, RSA was not measured so it is unclear how the prescribed training affected this performance parameter.

In competitive rugby players, 6 weeks of RSA training (RST) (3 × 6 reps of 40 m shuttle sprints departing every 20 s with 3 min rest between sets) twice a week (n=10), or RSA and resistance training (RT + ST) (6 × 6 sets of explosive squats at the load that maximised individual power output on a vertical vibration platform) once each a week (n=10) supplemented regular rugby training and games sessions [25]. Substantial improvements were recorded in RSA mean time (∼2 vs. ∼4 %), RSA percent decrement (∼26 vs. ∼23 %) and squat absolute output (∼5.0 % vs. ∼17 %) in RST and RT + ST respectively. However increases in RSA best

time (∼2.6 %) and squat power normalised to body mass (18.6 %) occurred only in RT+RST. These results again highlight the relationship between squat strength and maximal sprint performance reported previously [16] and provide support for a combination of both specific resistance and RSA training in a periodised team-sport conditioning programme.

Kotzamanidis et al. [27] recruited 35 competitive soccer players (∼17 y) with at least 4 y training experience and allocated them to 1 of 3 specific training groups: 1) combined RSA and strength training (CT) (n=12), 2) strength only (ST) (n=11) and 3) control group (n=12). Strength training was divided in to 3 sub-periods which consisted of 4 sets at an intensity of 8-RM, 6-RM and 3-RM for each sub-period respectively. The RSA training consisted of 4,5 and 6 maximal reps of 30 m respectively with 3 min rest between repetitions. In the CT group RSA exercise was completed immediately after resistance training. The control group performed no training during the experimental period. Training lasted for a total of 13 weeks. Significant increases were recorded in all 1-RM maximal strength exercises in CT and ST (half squat ∼17 %, step up ∼16 % and leg curl ∼16 %) with no differences between groups from pre to post training. However, SJ and CMJ improved by ∼7 % only in the CT group, and 30 m sprint time was also only improved (∼3.5 %) in the CT group. The combined lower-body strength and RSA exercise with prolonged rest intervals between reps (3 min) improved maximal strength and explosive movements whereas ST training resulted only in gains in maximal strength. This particular study provides evidence that RSA exercise may improve explosive power in soccer players, where as strength training may be predominantly limited to improving overall strength. Importantly, this study demonstrates the value of incorporating team-sport specific movements in to training programmes, and supports the inclusion of concurrent RSA and strength training for improvements in explosive power in team-sport players.

Fourteen recreationally active males (∼26 y) with 6 months previous resistance training experience, completed combined (CT) strength (3 sets of 6 RM at 85 % 1-RM for 6 upper and lower body resistance exercise) and RSA training (4-6 20 s modified Wingate cycle ergometer sprints, that was previously shown to increase aerobic capacity in 12 weeks) 4 × a week or strength only training (ST) 2 × week for 12 weeks [28]. RSA had no detrimental effect on strength performance, as 1-RM bench press and back squat increased significantly with no difference between training groups. However, as both groups performed the same volume of strength training, it also appears that the sprint training had no additive effect on maximum strength. Surprisingly, there were no significant differences between the groups for peak, average power and fatigue index during the Wingate test even though one group had specifically trained using Wingate sprints for 12 weeks. Only average power during

the Wingate test improved (∼9 %) over time for both groups. As expected, the CT group increased their $\dot{V}O_2$max from 40.9 ± 8.4 to 42.3 ± 7.1 (mL·kg^{-1}·min^{-1}), while the ST group showed no change. Longer duration (> 20 s) repeated sprints have previously been shown to be as effective as traditional endurance training in improving aerobic performance [128] so the increases in $\dot{V}O_2$max in this study are not surprising. However RSA or ground-running sprint performance was not measured. Further, sprints on a cycle ergometer are not specific to the mechanics of running sprints or team-sport performance and therefore protocols that consist of maximal running sprints may be more beneficial and practical for team-sport athletes.

However, Wong et al. [22] were also able to show that repeated-sprints of 15 s duration combined with resistance training improved YYIRT performance in professional soccer players (∼25 y). The effectiveness of combining high-intensity interval training (HIT) and resistance training on measures of explosive power were tested in professional soccer players [22]. Thirty nine players were assigned to either a control group (CG) or an experimental group (EG) and participated in 8 weeks of regular soccer training, with the EG completing two additional resistance and HIT training sessions per week. Resistance training consisted of 4 sets of 6-RM of high-pull, jump squat, bench press, back half squat, and chin-up exercises. The HIT consisted of 16 intervals each of 15 s ground running sprints at 120 % of individual maximal aerobic speed interspersed with 15 s of rest. Strength training was performed in the mornings, while HIT was performed in the afternoon (∼5 hours after the morning session). Combined HIT and resistance training improved 1-RM back squat (123.0 ± 1.5 kg to 148.0 ± 1.9 kg) and 1-RM bench press (65.3 ± 1.5 kg to 70.4 ± 1.1 kg) and also increased vertical jump height (+ 2.5 cm) pre-post training. Combined HIT and strength training also had significant effects on the acceleration phase of a maximal 30 m sprint (-0.11 s), while 30 m sprint was improved by -0.12 s. Further both maximal aerobic speed (+ 0.5 km.h^{-1} $p \leq 0.05$) and YYIRT distance was increased by ∼300 m (∼20 %) which was higher than the 137 m (∼8 %) improvement caused by regular team-training in the CG.

The above two studies by Cantrell et al. [28] and Wong et al. [22] recorded the greatest gains in aerobic capacity, suggesting that longer duration sprints are required to improve aerobic capacity, due to the greater oxidative contribution during longer duration sprints. However, 'traditional' concurrent aerobic and resistance concurrent training have shown decrements in strength, hypertrophy and power gains [17–19], and RSA exercise has been shown to induce molecular adaptations similar to those elicited by traditional aerobic exercise [129]. Therefore, future research may aim determine if there are differences in the compatibility of resistance and RSA exercise between protocols with short predominantly anaerobic (< 5 s) and longer (greater aerobic contribution) (< 15 s) duration repeated-sprints.

Ross et al. [29] had the only study to compare the benefits of resistance only (RT, n=6) sprint (treadmill) training only (ST, n=9) or combined resistance and sprint (SRT, n=10) training found favourable improvements with SRT on both land and treadmill sprinting. As well, changes in maximal strength of the same magnitude as RT in male former or current competitive team-sport athletes (∼20 y) were observed. Sprint training was performed on a Woodway treadmill that allowed resisted sprint training (8-12 maximal sprints for 40-60 m at 0-25 % of each athlete's body mass with 2-3 min rest intervals) 2 × week for 7 week. Resistance training was completed 4 × week for 7 weeks and consisted of 9 upper and lower body resistance exercises of 3-4 sets × 6-10 reps with 2-3 min rest intervals. SRT group completed all RT and ST, with ST performed prior to resistance training as the authors wanted to avoid residual fatigue impairing sprint performance. The 30 m land sprint time decreased significantly (0.10 s) in SRT, while a trend for ST to decrease 30 m land sprint time (0.08 s) was also reported. 20 m treadmill sprint velocity increased in all groups but was increased by more in the SRT (∼8 %) and ST (∼5 %) groups compared with the RT group (∼1.6 %). All groups increased maximum squat strength (6.6-8.4 kg) with no differences between groups. This is the first study to show that resisted sprint training can be just as effective at improving 1-RM squat performance as traditional resistance exercise, indicating similarities in adaptations. This is an interesting finding, which could suggest that resisted sprint training could be an effective training protocol for athletes who are particularly short on time. However, again RSA performance was not measured and it is therefore unclear whether resisted sprint training would be as effective as standard maximal sprinting in improving this team-sport specific performance quality. Also, training an entire team using resisted treadmills may prove to be difficult to implement.

There was only one investigation not to report significant improvements in performance with concurrent training in-season, combined resisted agility and repeated sprint training in elite (training ∼10 h per week) female soccer players (∼19 y) for 10 weeks. Resisted agility exercises were completed in pairs using resistance bands attached at the waist. Twenty elite female soccer players compared the effects of adding 2 × strength (squats and leg extension/flexion, 2-3 sets × 5-8 RM) (n=10) or 2 × resisted agility (4 agility exercises: standing starts, 180 degree turns, forwards and backwards running in pairs connected with elastic belts) and concurrent RSA (2-5 sets of 4-5 40 m sprints at 90-100 % intensity with each sprint separated by 30 s, and sets separated by 10 min) trainings per week on agility, linear single sprint speed, vertical jump, RSA, and aerobic capacity. These trainings were performed alongside normal team soccer trainings [126]. The resisted agility and RSA group did not improve SJ, CMJ, RSA (7 × 30 m), or 40 m (acceleration or maximum speed phase) sprint performance or agility test performance. The strength training

group improved SJ performance (∼5.8 %) and both groups improved 20 m multi-stage shuttle run test score by ∼1 level but this was attributed to regular team soccer trainings. It is difficult to determine why this study, unlike the other studies reviewed, reported no performance improvements with concurrent training, particularly as no decrements in performance were detected. However, one suggestion could be that the exercise stimulus was not strong enough to elicit improvements in already well-trained female athletes. Therefore further investigations are pertinent to enable the design of a training regime that can improve (rather than maintain) team-sport specific performance variables in well-trained female athletes.

The effects of the intra-session sequencing of RSA and resistance exercise on team-sport performance variables has only been investigated once in the literature to date and was completed in semi-professional soccer players (∼25 y) from a Swedish Division 1 team [30]. McGawley et al. [30] had players complete 3 experimental led sessions per week for 5 weeks alongside 2 coach led sessions focused on tactical and technical elements of soccer during the pre-season [30]. The experimental led session consisted of 30 min of HIT (various RSA and high-intensity interval protocols including repeated 30-40 m sprints and 4 min intervals at 95 % HR max) a 5 min change over period and 30 min of strength exercises (gym-based of 2-3 sets × 5-10 reps with progressive overload from 75-90 % 1-RM and pitch based exercise for explosiveness, power and core development). Two groups completed the combined HIT and STR training, one group completed the exercises in the HIT-STR order (n=9) while the other group completed the exercises in the STR-HIT order (n=9). Each session was separated by 5 mins. Training of both orders significantly increased 10 m sprint, 6 × 30 m RSA, 40 m agility and YYIRT test performances improving by 1.8 (± 2.6), 1.3 (± 1.8), 1.0 (± 1.5) and 19.4 (± 23.4) %, respectively. Interestingly though, there were no significant differences between the changes in performance over time between the two different orderings of the exercises, which may suggest that the ordering of the exercises in this particular study did not differentially alter the physiological adaptation required for the performance improvements between groups. This is an intriguing finding as the only study to investigate the acute molecular profile of combined RSA and resistance exercise of different intra-session orderings suggested there may be an optimum structure for these two modes of exercise [72].

To examine the effect of intra-session order of RSA and resistance exercise on molecular signalling in skeletal muscle, Coffey et al. [72] had 6 male participants who were already completing regular concurrent resistance and endurance training participate in an acute concurrent exercise study. After a 10 h fast, participants arrived at the laboratory for resting skeletal muscle biopsies from the vastus lateralis. Following, participants completed either 8 × 5 reps of leg extension at 80 % 1-RM

which was followed by 15 min of passive rest and then 10 × 6 s maximal cycle sprints against 0.75 N·m torque·kg^{-1} with sprints separated by approximately 49 s active recovery or the vice versa. The authors reported that initial resistance exercise increased p70S6K phoshporylation (∼75 %), but this did not occur when resistance exercise was undertaken after sprints suggesting an attenuation of the anabolic response. Further evidence for attenuation of anabolic responses to resistance exercise by concurrent sprint exercise was seen through a decrease in IGF-I mRNA and an accompanying increase in Muscle RING-finger protein (MuRF) mRNA abundance following 3-h recovery. PGC-1α mRNA was increased following either order but was higher when sprints preceded resistance exercise (ES > 1.0). Lactate, pH and H^+ were similar following both orders of exercise, but lactate and H^+ were higher during resistance when preceded by sprints. In terms of performance, mean power generated in the RSA protocol was similar regardless of exercise order. The authors therefore tentatively suggested that RSA should be isolated from resistance exercise to eliminate interference effects of the RSA exercise on translation initiation signalling following resistance exercise [72]. However, as the repeated-sprints in this study were undertaken on a cycle ergometer, it is unclear how specific these molecular responses would be to running RSA exercise and thus team-sport athletes. Further, as the study by McGawley et al. [30] reported no differences in performance adaptation irrespective of the order of same-session HIT/RSA and resistance exercise, whether the molecular mileu to this type of exercise is important in performance adaptation in well-trained team-sport athletes is unclear.

Of the 10 studies reviewed (see Table 2.1) that looked at performance responses to concurrent RSA and resistance exercise from pre to post training, only one failed to report any significant increases in explosive power performance as assessed by jumping and maximal sprinting over 30 m [126]. Interestingly, this was also the only study to determine the effectiveness of concurrent resistance and RSA/agility type exercise in an elite female team-sport population. This suggests that specific research with females is perhaps an area of research that needs more attention. While research in male participants provides important information and knowledge about particular training paradigms, subtle differences between the sexes in exercise metabolism [130], hormonal responses [131], and maximal power development [132] may promote somewhat different adaptation profiles. Thus female specific physiological and performance responses to a combined RSA-resistance training complex are needed to ascertain the benefits for the growing female team-sport population.

Table 2.1: Summary of studies investigating concurrent repeated-sprint and resistance training.

Author	Participants	Intervention and training	Performance Outcomes		
Wong et al. [22]	n = 39. Male professional soccer players	8 week intervention period. Control group (CG) completed regular team trainings only. Experimental Group (EG) completed RT and HIT 2 × week additional to team trainings. RT = 4 × 6 jump squat, bench press, back half squat, chin ups (3 mins rest btwn sets) + 3 × 15 plyometric sit-ups throwing 3 kg medicine ball. HIT = 16 × 15 s intervals at 120 % maximal aerobic speed (15 s rest between sprints)	**EG**	**CG**	
			CMJ: ↑	↔	
			SJ: ↑	↔	
			0-10 m: ↑	↔	
			0-30 m: ↑	↔	
			RSA: ↭	↭	
			Squat 1-RM: ↑	↔	
			$\dot{V}O_2$max: ↭	↭	
			YYIRT: ↑	↔	
Shalfawi, S. [126]	n = 20. Elite female soccer players	10 week intervention period. RSA + Agility group 2 × week and RT group 2 × week additional to regular team trainings. RSA + Agility = resistance band sprinting in pairs with a band connected with belts around each others waste. RSA = 2-5 sets of 4 × 5 40 m sprints at 95-100 % intensity, sprints separated by 30 s rest. RT + 2-3 sets 5-8 RM of leg press, SJ, nordic hamstring, leg extension, cable hip flexion + extension	**RSA + Agility**	**RT**	
			CMJ: ↔	↔	
			SJ: ↔	↑	
			40 m: ↔	↔	
			20 m: ↔	↔	
			20 m fly: ↔	↔	
			Beep Test: ↑	↑	
			$\dot{V}O_2$max: ↭	↭	
			RSA: ↔	↔	
Kotzamandis et al. [27]	n = 35. Healthy male volunteers.	13 week intervention period, 2 × week. Combined resistance + speed programme group (RTS). Resistance only group (RT) and Control group (CT). RT = 4 × 3-8-RM. Speed = 4-6 max reps of 30 m with 3 min rest between sets.	**RTS**	**RT**	**CT**
			CMJ: ↑	↔	↔
			SJ: ↑	↔	↔
			DJ: ↑	↔	↔
			1-RM squat: ↑	↑	↔
			30m: ↑	↔	↔
			Leg Curls: ↑	↔	↔
			$\dot{V}O_2$max: ↭	↭	↭
			RSA: ↭	↭	↭
Ross et al. [29]	n = 25. Current or former competitive athletes in team-sports or track and field.	7 week intervention period, 2 × or 4 × week. Treadmill sprint group (ST). Resistance only group (RT) and combination (SRT). ST = 8-12 max sprints for 40-60 m at 0-25 % b/w 2 × week. RT = 2 lower and 2 upper body sessions a week, 8-9 exercises of 6-10 reps. SRT = All ST and RT sessions, ST completed prior to RT.	**ST**	**RT**	**SRT**
			30m (land): ↑	↔	↑
			20m (treadmill): ↔	↑	↑
			20m pp (treadmill): ↔	↔	↑
			1-RM squat: ↑	↑	↑
			$\dot{V}O_2$max: ↭	↭	↭
			RSA: ↭	↭	↭

Marques et al. [26]	$n = 52$. Competitive youth soccer players competing at national level	6 week intervention period, 2 × week. Plyometric and sprint training group (PST) and regular team training. Control group (CT) regular team trainings. PST = 2 legged jumps, 1 legged jumps, 1 legged quick hops all 3 × 20, 1 legged high jumps 2 × 8, sprint from standing 6 × 20 m, sprint from lying 5 × 20 m.		**PST** / **CT** CMJ: ↑ / ↓ 0-15 m: ↔ / ↔ 15-30 m: ↑ / ↔ 0-30 m: ↑ / ↔ $\dot{V}O_2$max: ⇜⇝ / ⇜⇝ RSA: ⇜⇝ / ⇜⇝
Tsmahidis et al. [23]	$n = 26$. Healthy junior basketball players	10 week intervention period, 2 × week. HIT resistance + max sprints group + regular team trainings (RTS) and control group (CT) + regular team trainings. RT = 5 × 5-8 RM half squat (90 s rest periods), S = 30 m maximal sprint after each resistance set.		**RTS** / **CT** CMJ: ↑ / ↔ SJ: ↑ / ↔ 0-10 m: ↑ / ↔ 0-30 m: ↑ / ↔ Squat 1-RM: ↑ / ↔ $\dot{V}O_2$max: ⇜⇝ / ⇜⇝ RSA: ⇜⇝ / ⇜⇝
Cantrell et al. [28]	$n = 14$. Recreationally active men.	12 week intervention period, 2 × week. Resistance training group (RT) and combined training group (CT) 2 × week (72 h rest) performed resistance and sprint exercise for total 4 × week (24 h rest). RT = 3 × 4-6 reps at 85 % 1-RM (2 min rest intervals) of back squat, bench press, leg extension, leg curl, pull-down + shoulder press. Sprint = 4-6 modified 20 s Wingates on a cycle ergometer.		**CT** / **RT** Squat 1:RM: ↑ / ↑ Wingate pp: ↔ / ↔ Wingate av p: ↑ / ↑ Wingate Fatigue: ↔ / ↔ $\dot{V}O_2$max: ↑* / ↑ RSA: ⇜⇝ / ⇜⇝
Alonso et al. [24]	$n = 13$. Youth basketball players with no prior resistance training experience.	6 week intervention period, 2 × week. Control group (CG) completed regular team trainings only. Experimental group (EG) completed sprint exercise + half squat in weeks 1-3 and half squat + plyometrics in weeks 4-6. Half squats = 4-5 × 6-10 reps at 85 % 1-RM (3 min rest period). Sprints = marching A drill, skipping A drill resisted sprint all 2 × 20 m, T Test × 6 + 7 × 40 m shuttle sprint with 20 s between sprints. Plyometrics = Stiffness jumps .Jump squats, Hurdle jumps + DJ all × 6		**EG** / **CG** CMJ: ↑ / ↓ SJ: ↑ / ↓ DJ: ↑ / ↓ Jump Squat: ↑ / ↔ Agility: ↑ / ↔ YYIRT1: ↑ / ↔ $\dot{V}O_2$max: ↑ / ↔ RSA: ↑ / ↔

McGawley et al. [30]	n = 18. Semi- and fully professional soccer players.	5 week intervention period, 3 × week. Two groups who completed regular team trainings + 3 additional experimental sessions consisting of HIT + repeated sprints and resistance trainings separated by 15 mins, groups completed the exercise in opposite orders (HIT:RT and RT:HIT). HIT consisted of a combination of 5-60 s sprints with 10-90 s rest, 4 × 4 min intervals @ 90-95 % HR max and 4 × 6-8 30 or 40 m sprints with 30 s rest intervals. RT = 2-3 × 5-10 of a number of exercises including but no limited to: squats, nordic curls, core rotations, barbell rowing, lunges, hamstring kicks, bounding jumps.	**HIT:RT** / **RT:HIT** CMJ: ↑ / ↑ SJ: ↑ / ↑ Squat 1-RM: ↑ / ↑ 0-10 m: ↑ / ↑ Agility: ↑ / ↑ YYIRT2: ↑ / ↑ Hanging situps: ↑ / ↑ $\dot{V}O_2$max: ↑ / ↑ RSA: ↑ / ↑ RSA Fatigue: ↑ / ↑ No differences between groups.
Suarez Arrones et al. [25]	n = 20. Male rugby players.	Repeated sprint training only (RST) group 2 × week + regular team trainings and combined RT and RST (RT+RST) 1 × each per week for total of 2 × sessions. RST = 6 × 40 m shuttle sprints with 20 s between reps, 4 min rest between sets. RT + 6 × 6 sets of explosive squats at the load that maximised power output on a vibration platform, 20 s rest between sets.	**RT + RST** / **RT** Squat power rel. b/w: ↑ / ↔ Squat power abs: ↑ / ↑ $\dot{V}O_2$max: ⇜⇝ / ⇜⇝ RSA mean time: ↑ / ↑ RSA best time: ↑ / ↑ RSA fatigue time: ↑ / ↑
Coffey et al. [72]	n = 6. Healthy males experienced with concurrent endurance and resistance training	Randomised cross-over design. Participants completed 2 trials completing resistance and repeated sprint exercises in both orders (RSA:RT and RT:RSA). RT = 8 × 5 reps at 80 % 1-RM of leg extension. RSA = 10 × 6 s maximal cycle sprints at 0.75 Nm torque-1 kg-1	RT increased S6K phosphorylation (75 %), but not when undertaken after sprints. Exercise decreased 1GF-1 mRNA following 3 h recovery (50 %) independent of order. PGC-1 α increased in the RT:RSA order. RSA appear to promote the overriding acute exercise induced response in a concurrent RSA:RT regime.

RT = Resistance exercise/training. HIT = High intensity interval training. RSA = Repeated sprint ability. S6K = Ribosomal protein S6 Kinase. IGF-1 = Insulin-like growth factor 1. PGC-1α = Peroxisome proliferator-activated receptor gamma co-activator 1-alpha. (↑ = Significant increase, ↔ = No change, ↓ = Significant decrease, from pre-post training) ⇜⇝ = Not measured.

Of the studies that compared combined RSA and resistance exercise with resistance only exercise, combined training reported similar increases in maximal strength and explosive power to the resistance only groups [27–29], suggesting that molecular interference may not have been influential in these studies, or that our understanding of the contribution of molecular signalling to adaptation in combined exercise studies is unclear. In progression from the work by Coffey et al. [72] investigating acute signalling responses prior to training and following differing durations of training of concurrent RSA and resistance exercise may elucidate how the signalling response adapts over time and may provide information as to how important molecular adaptation is to improving performance with this type and mode of exercise.

Also, it may be that the increases in maximal strength, explosive jumps, acceleration and maximal sprint speed over 10-30 m in team-sport athletes in the current studies were predominantly due to neural adaptations, that as a result of high load strength training contractile efficiency and/or mechanical characteristics were improved. While not measured in any of the reviewed concurrent RSA and resistance studies, maximal strength training and combined aerobic and resistance exercise have been shown to enhance neuromuscular activity of the working muscle [46, 92].

As well, increases in body mass by increasing skeletal muscle may not be advantageous or desirable for all team-sport players of different codes, or even different positions within the same code, particularly for non-contact sports. Therefore making gains in strength through predominantly neuromuscular improvements with minimal increases in mass may be preferential. Whereas the opposite may be true for rugby union and league players who are required to perform a number of collisions and tackles both offensively and defensively and therefore greater mass would create a greater sprint momentum [13]. The strength and conditioning practitioner may therefore choose to alter the resistance protocol based on these requirements. However, lower load-higher rep resistance training combined with RSA training has yet to be investigated in team-sport athletes, and it may be that this combination may be more susceptible to a molecular interference effect due to the reliance on skeletal muscle hypertrophy for performance adaptation.

Conclusions

Concurrent RSA and high-load, low-repetition resistance training appear compatible in their adaptations and the combination of these exercise modes appear to be effective at increasing explosive movements deemed important for team-sport performance, specifically in males. Approximately two concurrent RSA and high-load, low repetition resistance sessions per week appear to be a sufficient stimulus to elicit sig-

nificant increases in team-sport performance variables. However, there is a paucity of data in female team-sport athletes and this should be addressed in future studies to ensure optimal training strategies for the growing female team-sport population. Also, the physiological cost of team-sport specific RSA and resistance exercise is yet to be investigated. Analysing the immunoendocrine, and molecular responses to this type of exercise may provide valuable information as to how stressful the exercise is on the athletes and thus how well athletes can tolerate the exercise while also allowing for the determination of how the intensity of training compares to match-play. The combination of these variables will likely provide valuable information as to how much rest and recovery athletes require between training sessions in order to be in optimal physiological condition for match day.

CHAPTER 3

Exercise induced systemic and skeletal muscle cytokine responses in healthy populations: a review

Abstract

The cytokine response to exercise has become a recent focus of attention in the sport sciences. Strenuous exercise is a potent stimulus for the systemic and local production and circulation of pro- and anti-inflammatory responsive cytokines and chemokines. The inflammatory cascade to exercise is led by the immuno-modulator IL-6 which precedes the elevation of IL-10 and other cytokines. In contrast to sepsis or infection, systemic TNF-α and IL-1β present little or no change to exercise, while increases in these pro-inflammatory cytokines have been observed in the skeletal muscle following exercise. The cytokine response appears to be tightly related to the duration and intensity of exercise and working muscle mass. Moreover, skeletal muscle has been shown to express a number of cytokines including; IL-6, IL-8, IL-15 and TNF-α. In particular, IL-6 is regulated by skeletal muscle in response to contraction during exercise at both the mRNA and protein levels and appears to be heightened during states of low muscle glycogen. The effect of training on the acute exercise induced cytokine response to exercise remains largely equivocal and requires further investigation in healthy athletic populations. It appears that the pro-and anti-inflammatory cytokine response is balanced between the local pro-inflammatory response and the systemic anti-inflammatory responses. The exact contributions of cytokines to exercise induced muscle damage and adaptation, mobilisation of circulating immune cells, and metabolism remain to be fully elucidated.

Introduction

The cytokine response to exercise has become a recent focus of attention in the sport sciences. Cytokines are small peptides or cell-signalling proteins that are secreted by a number of cells that mediate the inflammatory response to exercise. Cytokines are loosely classed in to two categories: pro-inflammatory (such as tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1, 1L-1β) which work to amplify inflammatory reactions and anti-inflammatory (such as IL-10, and IL-1 receptor antagonist (IL-1ra)) that act to suppress the effects of the pro-inflammatory cytokines and reduce inflammation. IL-6 is now widely accepted as an immuno-modulator or inflammation responsive cytokine [133] that mediates the cytokine cascade response to exercise [134,135]. Further, in lean athletic individuals skeletal muscle comprises in excess of 40 % of total body mass equating itself to the largest organ in the human body. Therefore the finding that skeletal muscle can act as a secretory organ stimulated and controlled by contraction [32] has cast open a large and diversified research field in sport science. It is well established that cytokines play a focal role in both initiating and mediating the acute inflammatory response to exercise of different modes and durations [see review, [134]] while the long term effects of exercise training and adaptation on the acute cytokine responses in healthy adult populations are less widely researched. IL-6 has received the most attention of the exercise induced cytokines and as such will have a particular focus in this review. Therefore the present review 1) summarises the roles of IL-6 during and following exercise, 2) summarises the acute and chronic systemic and local cytokine response to exercise in healthy adult humans, 3) discusses the role of chronic exercise training and training status on regulating the cytokine expression at rest and post acute exercise in healthy adult populations and 4) discusses the role of sex in regulating the acute post-exercise cytokine response. While this review will focus on previous works in healthy adult populations, references to rodent studies may occur where human studies are scarce or to help explain possible mechanisms of action.

Interleukin-6

Interest in IL-6 and its role during exercise has increased since 2000 when it was shown that exercising skeletal muscle can release IL-6 into the circulation during exercise [136]. Dramatic increases in circulating levels of IL-6 were reported up to 50 and 100 fold following competitive marathon running [137] (marathon, $\sim$ 3 h 20 min duration) and extreme long endurance running (160 km) [138]. However, increases between 1-20 pg/mL are more consistently reported with exercise of shorter durations ($<$ 60 mins) [37, 37, 139–141] or lower intensity ($<$ 70 % $\dot{V}O_2$peak) [140,

142–144], intermittent in nature (team-sport/soccer) [145] or knee extensor exercise [146–148]. The peak concentration reached for IL-6 during exercise appears to be heavily dependent on duration of exercise with a review showing more than 50 % of the variation in IL-6 following exercise can be explained by exercise duration alone [31]. However, exercise intensity and the mass of skeletal muscle mass recruited have also been shown to be indicative of IL-6 response [32] and as such needs to be considered when interpreting IL-6 studies.

Skeletal muscle produces and releases interleukin-6

Skeletal muscle was first quantified as a major source of IL-6 when it was shown that human myoblasts [149] and cultured human myotubes expressed IL-6, and that cultured human muscle cells under mechanical strain increased the presence of IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [150]. Skeletal muscle IL-6 mRNA and the muscle transcriptional rate for IL-6 increases following exercise in humans and is heightened in a diminished muscle glycogen state [146].

Steensberg et al. [136] was the first study to discover an increase in IL-6 net release from the contracting skeletal muscle in exercising humans. Steensberg et al. [136] were able to demonstrate through simultaneous arteriovenous measurements of IL-6 concentration and concurrent measurement of blood flow with the doppler technique, that large amounts of IL-6 are released from the contracting muscle into the systemic circulation. They also suggested that the magnitude of the IL-6 release from the working muscle could account for the exercise induced increases in plasma IL-6, further suggesting that skeletal muscle may be the predominant source of exercise induced IL-6 production. In support for the idea that the skeletal muscle IL-6 release is likely responsible for the plasma IL-6 elevations during exercise, IL-6 mRNA level in monocytes was shown not to increase as a result of bicycle exercise [151]. Starkie et al. [152, 153] confirmed this at the protein level, demonstrating on two occasions that circulating monocytes do not contribute to the elevations in circulating IL-6 during submaximal running (competitive marathon race) and cycling (2h at 70 % $\dot{V}O_2$peak) exercise. Therefore, it appears that monocytes are not the source of increased IL-6 during exercise, suggesting skeletal muscle may well be the primary source. However, MacDonald et al. [142] disputed these results, arguing that the release of IL-6 from muscle could not alone explain the elevations in plasma IL-6 during exercise.

It was further shown that the production and release of IL-6 comes from the actual contracting fibres [40]. In rodents, IL-6 was produced in skeletal muscle following involuntary contraction via electrical stimulation. IL-6 mRNA was re-

vealed in isolated myofibres following the electrically stimulated contractions, while no IL-6 mRNA was found in the myofibres in the contralateral leg that did not exercise. Both eccentric and concentric contractions produced similar increases in IL-6 mRNA [40], suggesting that IL-6 production in exercised muscle is not necessarily related to muscle damage.

Small amounts of IL-6 protein have been detected in skeletal muscle by the use of high sensitivity immunohistochemistry [154]. Open human muscle biopsies taken pre and post 2 h cycling exercise analysed via immunohistochemistry, and hybridisation for IL-6 protein and IL-6 mRNA content found significant elevations in IL-6 protein and IL-6 mRNA after exercise [154], therefore demonstrating that not only is IL-6 gene expression increased following exercise, but functional protein is also increased.

Improvements in technology have made the measurement of skeletal muscle cytokine protein abundance easier to measure, with the following two studies measuring protein concentration in muscle homogenates (prepared as for western blot) with Bio-plex suspension array. Recently, the protein expression of IL-6 has been measured in skeletal muscle following unilateral knee extensor exercise (3×12 reps at maximum with 2 min rest) with a $\sim$4 fold increase in expression of IL-6 at 2 h post-exercise [155] in 8 resistance untrained men, and a $\sim$ 17.5 fold increase at 3 h post-exercise [156] in 13 resistance untrained healthy males following the same exercise protocol. Differences in the biopsy timing may provide a reasoning for the differences in fold change of IL-6 following the same exercise protocol. The optimal timing for skeletal muscle biopsies post-exercise for the measurement of inflammatory cytokines is presently unknown.

Interleukin-6 and its possible roles in response to exercise

IL-6 was originally thought to be released from the muscle in response to inflammation caused by muscle damage; however studies in the last 15 years have shown that muscle damage is not the only or necessarily major reason for its release [see reviews: [32, 33]]. Following cycling exercise (known to produce minimal muscle damage due to the lack of an eccentric component) the rise in exercise induced IL-6 has been shown to be similar to that induced by running [148]. Similarly, following repeated bouts of eccentric exercise, inflammation (measured as delayed onset muscle soreness and serum myoglobin levels) appears to be reduced with subsequent bouts, but the rise in IL-6 remains unchanged; providing evidence that muscle damage does not appear to be a pre-requisite for IL-6 release [157]. Another important finding is that IL-6 production in skeletal muscle myocytes is independent of and in the absence of TNF-α [158], a contrast to inflammatory conditions (like sepsis or

infection) in which TNF-α regulates IL-6 and other inflammatory cytokines. This suggests that IL-6 may play different or simultaneous roles in response to exercise. One such role that has garnered a lot of support is a role for IL-6 in exercise metabolism

Interleukin-6 and glucose metabolism

Evidence for IL-6 release from the skeletal muscle suggests that the rise in IL-6 during exercise is dependent on the duration of exercise and the reduction in muscle glycogen stores [33], leading to the hypothesis that IL-6 may be released from the skeletal muscle and into the systemic circulation as a marker of 'energy crisis' in order to stimulate hepatic glucose output [33, 159] (Figure 3.1). In support of this theory, in 1997, it was first shown that the systemic IL-6 response to exercise was attenuated upon the ingestion of carbohydrate (CHO) [160], and this effect has continued to be consistently reported during endurance exercise [36, 37, 41, 161, 162]. While CHO ingestion has also been shown to reduce the plasma IL-6 response to soccer-specific high-intensity intermittent (HIT) running [163].

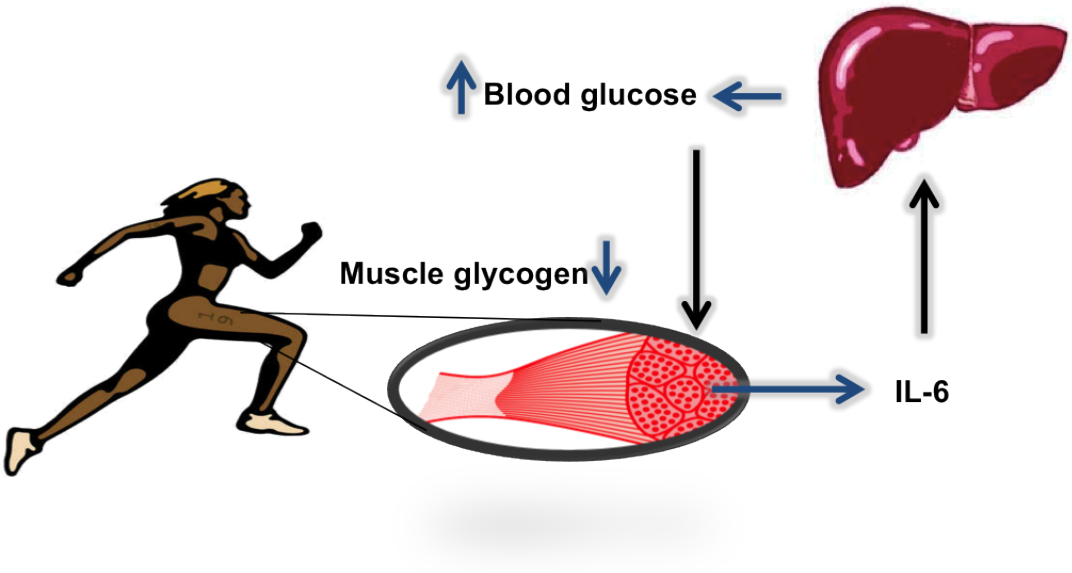


Figure 3.1: The role of skeletal muscle produced and released IL-6 in glucose metabolism during exercise.

Gleeson and Bishop [164], showed that when exercise is commenced in a glycogen-depleted state the systemic IL-6 response to exercise is elevated compared with beginning exercise with plentiful glycogen stores. A similar experiment was

carried out by Keller et al. [146], which included the measurement of IL-6 mRNA. Six untrained males completed two trials of dynamic leg extensor exercise (180 min at 60 % of their maximum 2-min work load) with either normal pre-exercise glycogen levels, or with glycogen levels ∼40 % lower than normal pre-exercise levels. Plasma IL-6 was significantly higher at the end of exercise (∼10 pg/mL vs. ∼6 pg/mL) in the low glycogen trial. IL-6 mRNA also followed a similar pattern, increasing by more than 100 fold in the low glycogen trial and by only 30 fold in the control trial. The authors concluded that they had provided support for the hypothesis that IL-6 is produced by contracting fibres when glycogen levels are significantly reduced, possibly to signal to the liver to increase glucose production. Similarly, MacDonald et al. [142] found that in endurance trained males, IL-6 release from the exercising leg (60 mins cycling at 70 % $\dot{V}O_2$peak) occurred after ten minutes in a pre-exercise glycogen depleted state (∼17% of the glycogen loaded state) while no IL-6 release from the leg was seen when exercising in a pre-exercise glycogen loaded state. Though, in contrast to the Gleeson and Bishop [164] and Keller et al. [146] studies, plasma IL-6 increased similarly between trials, which contests the idea that the skeletal muscle is the predominant source of IL-6 during exercise.

A study by Helge et al. [148] determined via direct arteriovenous measures that IL-6 release from contracting skeletal muscle is positively related to both exercise intensity and the glucose uptake which was also positively correlated with plasma adrenaline concentration. Again demonstrating a role for IL-6 in glucose metabolism during exercise. In human muscle biopsies, IL-6 protein and IL-6 mRNA content have been shown to be elevated following 120 min of cycling exercise (at a power output equivalent to ∼55 % of their individual % $\dot{V}O_2$peak) and the distribution of IL-6 found to favour those fibres that were also high in glycogen content, with the majority being type II fibres [165].

Interestingly, CHO supplementation appears to lower only metabolically induced systemic IL-6 during exercise, as increases in IL-6 following eccentric exercise specifically designed to cause muscle soreness and inflammation (high-force eccentric contractions of the elbow flexors) are not attenuated with CHO ingestion [166, 167]. CHO ingestion may even increase inflammation stimulated systemic IL-6 concentrations and IL-6 mRNA expression 24 h following intense eccentric contraction [168, 169]. Again, in support of the idea that CHO ingestion affects metabolically induced IL-6 expression, it has been found that CHO ingestion during exercise attenuates only the plasma IL-6 concentration, and the release of IL-6 from the contracting muscle, while at the same time CHO ingestion has not been shown attenuate intramuscular expression of IL-6 mRNA following running and cycling exercise (60 min) [36,37]. This suggests that the release of IL-6 from skeletal muscle is prevented when blood glucose levels are sufficient.

Further, a study by Febbraio et al. [35] using stable isotope 6,6 2H2 glucose infusion to measure endogenous glucose production, found higher glucose rate of appearance, rate of glucose disappearance and metabolic clearance rate (MCR) during 120 min of high-intensity bicycle exercise (70 % $\dot{V}O_2$max) compared with low-intensity bicycle exercise (40 % $\dot{V}O_2$max). However when participants completed the low intensity exercise with rhIL-6 infusion at levels mimicking those seen in the high intensity exercise (∼10 pg/mL), glucose rate of appearance, disappearance and MCR were all higher than when lower intensity exercise occurred in the absence of rhIL-6 infusion. This data provides further evidence of a real role of IL-6 in maintaining glucose levels during exercise [35]. Interestingly, at rest acute administration of rhIL-6 in either what the authors suggested as high (319 pg/mL) or low (143 pg/mL) doses (however, in terms of exercise, these concentrations are very high and not representative of normal responses) did not promote an increase in glucose production or disposal in healthy humans [170,171], suggesting that there may be an 'exercise factor' released during contraction that acts as a synergist with IL-6 to control glucose metabolism during exercise. Interestingly, the low exercise trial of Febbraio et al. [35] detailed above, did not stimulate endogenous production of IL-6, but during infusion of rhIL-6, glucose rate of appearance and disappearance was higher, suggesting that the synergistic 'exercise factor' may already be present in the circulation during even low-intensity exercise but is perhaps not potent enough to stimulate elevations in systemic IL-6.

While not a human exercise study, a recent study using IL-6 knockout (KO) mice provides contrasting evidence to challenge the role of exercise induced IL-6 in glucose metabolism. O'Neill et al. [172] showed IL-6 is not essential for glucose uptake in skeletal muscle during exercise. This study found that body mass, energy intake and output, substrate utilisation and glucose and insulin tolerance did not differ between IL-6 KO mice and controls at rest. Further maximal exercise capacity was similar. During experimental procedures the mice ran for 40 min at 70 % maximal running speed which significantly elevated plasma IL-6 (∼39 pg/mL) in control mice. $\dot{V}O_2$ and respiratory exchange ratio (RER) were not different during steady state exercise between control and IL-6 KO animals, suggesting IL-6 may not regulate substrate utilisation during exercise. In addition and more importantly, plasma glucose concentrations were similar between groups at the end of exercise and skeletal muscle glycogen was also comparable between groups. Glucose clearance in tibialis anterior, extensor digitorum longus and soleus was not different, suggesting IL-6 does not influence glucose uptake in skeletal muscle during exercising conditions. In light of these findings in mice, further human experimental trials are required to substantiate the role of IL-6 in glucose metabolism and determine the biological mechanisms by which it acts.

Some evidence is available in humans that supports a role for IL-6 glucose uptake to be mediated by 5-AMP-activated protein kinase (AMPK). *In vitro*, the effects of 10-120 min of exposure to supra-physiological levels (1-100 ng/mL) of recombinant mouse IL-6 on glucose uptake in L6 myotubes were abolished in cells affected with an AMPK dominant-negative construct [173]. In a human exercise study, 8 healthy well-trained men completed 60 min of cycling at 70 % $\dot{V}O_2$peak in either a glycogen-depleted or a glycogen-loaded state [142]. IL-6 was released at 10 min in to exercise from the leg in only the glycogen-depleted trial despite similar increases in plasma IL-6 in both trials after 60 min of exercise. α2-AMPK activity increased in the glycogen depleted trial with individual levels correlating with individual levels of IL-6 release from the leg (r = 0.87) suggesting either a role for AMPK in the release of IL-6 from the contracting muscle or a role for IL-6 in the activation of AMPK during exercise [142]. An association with AMPK during exercise may provide support that IL-6 acts as an energy sensor during exercise as AMPK is activated in response to decreases in cellular energy states mirrored by increases in the AMP:ATP ratio [174].

Interleukin-6 and satellite cell proliferation and differentiation

As well as the IL-6 proposed role in inflammation and exercise regulated metabolism, IL-6 has also been implicated in skeletal muscle hypertrophy and repair via satellite cell proliferation and myogenic differentiation mediated through the IL-6 receptor (IL-6R) and glycoprotein 130 (gp-130) and the janus kinase (JAK) and signal transducers and activators of transcription (STAT) pathway [175,176]. Serrano et al. [177] were the first researchers to suggest a role for IL-6 in skeletal muscle hypertrophy. They were able to demonstrate using IL-6 KO, that when hind limb muscle removal was performed the typical compensatory hypertrophy response was in fact blunted. Further, studies have demonstrated an up-regulation of JAK/STAT signalling in the skeletal muscle during recovery from resistance exercise [156, 178], and of the STAT isoforms, STAT3 has been demonstrated to be most critical for satellite cell proliferation and differentiation via translocation to the nucleus and the transcriptional up-regulation of c-myc and cyclin D1. It has also been reported that a STAT3-MyoD complex can have a stimulatory effect on myogenic differentiation [179].

An acute resistance exercise session (3 sets of 12 maximal reps of maximal single-legged isokinetic leg extension) in untrained males caused the transient localisation of STAT3 to the nucleus and paralleled phosphorylation of STAT3 (Ty 705) at 2 h post-exercise [178]. Also peaking at 2 h post-exercise was the mRNA expression of STAT3 regulated transcriptional factors C-FOS, transcription factor jun-B (JUNB), c-MYC and vascular endothelial growth factor (VEGF). Suppressor of cytokine signalling 3 (SOCS3) was also elevated post-exercise [178]. In a similar

study by the same author (Trenerry et al.) [156] IL-6 and STAT3 protein concentration were measured in skeletal muscle prior to and following 12 weeks of resistance training in active but untrained adult males. IL-6 was increased 17.5 fold at 3 h post-exercise, while STAT3 phosphorylation increased 12.5 fold 3 h post-exercise with these responses apparently unaltered by training. Similarly, mRNA expression of c-myc, c-FOS and SOCS3 was increased following exercise but remained unaltered with training as was the negative regulating factor SOCS3 [156]. The results from these two studies led the authors to suggest that due to the preservation of STAT3 signalling with prolonged training, it may be essential to the adaptive and remodelling responses of skeletal muscle to acute resistance exercise.

Interleukin-6 and the systemic inflammatory response to exercise

Studies in athletically trained and untrained humans reveal that a number of pro- and anti-inflammatory cytokines appear to be regulated by exercise within the systemic circulation [134, 180]. IL-6 is known as the initiator protein for the anti-inflammatory pathway activated in response to exercise [133]. IL-6 has been shown to induce the release of anti-inflammatory cytokines (such as IL-10 and IL-1ra) rather than acting in an anti-inflammatory manner itself and as such has been termed an 'immuno-modulator' [133]. This is in contrast to the pronounced pro-inflammatory response activated in consequence to infection or sepsis which is characterised by marked increases in circulating TNF-α and IL-1β [181].

During exercise IL-6 precedes the rise in IL-10 and IL-1ra, supporting the idea that muscle derived IL-6 is the initiator of the anti-inflammatory response [182]. Similarly, infusion of rhIL-6 (corresponding to plasma levels of $\sim$140 pg/mL) at rest mimics the exercise response by stimulating increases in IL-1ra and the anti-inflammatory cytokine IL-10 in plasma while also increasing the catabolic stress hormone cortisol [183]. IL-10 has a suppressive effect on inflammatory cytokine macrophage functions, causing the inhibition of Type-1 cell cytokine production including IL-1 and TNF-α and chemokines IL-8 and macrophage inflammatory protein (MIP) from activated human monocytes [184,185]. IL-1ra is more specialised in that it inhibits signal transduction of IL-1α and IL-1β [186] which activate macrophages. IL-6 also appears to be able to suppress the production of TNF-α which is a pro-inflammatory cytokine that induces local tissue inflammation. In monocytes, IL-6 is shown to inhibit production of both pro-inflammatory cytokines TNF-α and IL-1β [187], which may explain why there are only mild increases seen in circulating TNF-α and IL-1β with exercise. The suppressive effects of IL-10 and IL-1ra on typical pro-inflammatory cytokines suggests that the cytokine response to exercise is likely a balance between these pro- and anti-inflammatory mediators, likely to ensure essential repairative processes are carried out.

Studies investigating the acute systemic inflammatory response during exercise highlight that pro-inflammatory cytokine production is generally well balanced and/or counter-acted by the elevation of IL-6 and anti-inflammatory cytokines such as IL-10 and IL-1ra. TNF-α and IL-1β appear to be only significantly elevated during strenuous high-intensity or metabolically demanding exercise and can be counterbalanced by specific cytokine inhibitors IL-1ra, sTNF-r1 and sTNF-r2 [133]. The magnitude of change in systemic cytokine concentrations varies markedly between cytokines, with IL-6 reported to have changes in excess of 100 fold after very prolonged endurance running exercise [137], but with changes in the range of 2-20 fold more consistently reported [36,37,145,146,182] following exercise, while plasma concentrations of IL-1β and TNF- α tend to increase 1-2 fold [133, 145].

Due to the expense and limitations of previous methods for measuring circulating cytokines, the majority of studies have analysed only a small number of cytokines within the circulation, most frequently IL-1β, TNF-α, IL-10 and IL-6 [135, 180]. With the introduction of bead based multiplex assays the ease and sensitivity of measurement has drastically improved and there is now the ability to perform up to 50 tests in a single biological sample. Therefore, future studies should aim to investigate the effects of exercise on a larger array of inflammatory cytokines that will provide a greater insight in to the complexities of and the contribution of the inflammatory system to the exercise induced response.

Acute systemic and local cytokine responses to exercise

The role of individual pro- and anti-inflammatory cytokines in response to exercise in healthy adult populations are outlined in detail below. The over-arching function of inflammatory cytokines in response to acute exercise is summarised in Figure 3.2.

Interleukin-10

Circulating IL-10 is closely related to the anti-inflammatory regulatory effects of IL-6, as IL-6 appears to stimulate IL-10, IL-1ra and soluble TNF receptors during exercise [183]. Few studies have specifically investigated the IL-10 response to exercise, instead due its known regulation by IL-6 it is often analysed alongside other cytokines as a measure of the inflammatory response to exercise stimuli. Therefore IL-10, like IL-6 has been elevated by exercise of various intensities and durations [34, 38, 133, 137, 145, 188]. Specifically IL-10 is increased following exercise of intensities (>60 mins of running at 85 % $\dot{V}O_2$peak [34], HIT intermittent exercise

during soccer [145]), durations (>2.5 h, marathon running [133, 182]) and muscle contractions (maximal eccentric exercise of the upper or lower body major muscle groups [38, 188]) likely to create disturbance in skeletal muscle or metabolic stress. IL-10 may then induce suppressive effects on macrophage functions [189] and suppress the pro-inflammatory response through the up-regulation of anti-inflammatory cytokines [190].

It was demonstrated for the first time by Della Gatta et al. [155] that acute resistance exercise (3 × 12 sets of maximal isokinetic knee extension at a constant speed of $60°s^{-1}$) can significantly increase the protein expression of IL-10 in skeletal muscle (∼1.12 fold), though this response was far more modest than the increases seen in pro-inflammatory cytokines (IL-8 ∼28 fold, MCP-1 ∼9 fold). The finding of IL-10 within skeletal muscle after exercise may suggest a role in the regeneration and adaptation of skeletal muscle following exercise, however results are preliminary and more research is required before the role of IL-10 in skeletal muscle can be clarified.

Interleukin-8

IL-8 is a chemokine that is predominantly produced by macrophages and synthesized by endothelial cells specifically targeting leukocytes [191]. IL-8 belongs to a large family of cytokines being part of the CXC subdivision of chemokines. IL-8 has two primary functions; during inflammation IL-8 is a neutrophil chemotactic or chemoattractant factor causing target cells, predominantly neutrophils to migrate towards the site of damage [192]. Independent of this role, IL-8 also promotes angiogenesis. To induce angiogenesis, IL-8 binds to the CXC receptor-2 (CXC-2) located within human microvasculature endothelial cells [193, 194].

Long endurance type exercise (>120 min duration) has been shown to elicit significant increases in plasma IL-8 [134,195–197], and small increases have been seen in plasma IL-8 following an elite female soccer match [145]. However, investigations designed with moderate concentric based only exercise such as cycling (60 min at 70 % $\dot{V}O_2$max) [39] and rowing (2 h on water training) [198] do not appear to influence plasma IL-8 concentration. In contrast an exhaustive incremental cycling protocol was able to increase plasma IL-8 concentrations by ∼35 % in trained and ∼50 % from pre-exercise values in untrained participants [199]. Another study investigating the effects of acute and chronic high intensity interval training (5 × 3 min bouts at 90 % $\dot{V}O_2$max on a motorised treadmill) in recreationally active males found an acute HIT session increased plasma IL-8 concentration and that this response was not attenuated by training (2-4 times per week for 6 weeks) [139]. Similarly, high intensity interval training in males induced modest increases in serum IL-8 (six HIT sessions at 8 - 12 intervals; 60-second intervals, 75-second active rest at a power

output equivalent to 100 % of their predetermined $\dot{V}O_2$max) but this response was also unaltered by 2 weeks of training [200]. These data suggest that exercise with some eccentric contribution that may induce muscle damage are required to induce a significant systemic IL-8 response. The acute post-exercise systemic IL-8 response is most likely owing to an inflammatory response with IL-8 acting in its role as a chemokine to attract neutrophils to the site of injury or damage.

Skeletal muscle has also been shown to express IL-8 in concentric and eccentric contracting fibres [39, 155, 201–203]. An *in vitro* study by De Rossi et al. [149] found that muscle cells had the capability to express both mRNA and protein expression of IL-8, and this has been confirmed in a number of exercise studies [155, 201–203]. mRNA expression of IL-8 has been shown to be markedly increased following resistance exercise (3 × 10 sets bilateral knee extension at 70 % 1-RM [202], 2 h of 10 exercises, 4 × sets each × 10 reps [201]) and both short and long duration running exercise (30 min treadmill running at 70 % $\dot{V}O_2$max [202] or 3 h at 70 % $\dot{V}O_2$max [204]) peaking between 4 and 8 h post-exercise. Following running [204] but not resistance [201] exercise IL-8 mRNA expression was attenuated by the ingestion of CHO (12 ml/kg, 6.4 % CHO, ∼64 g total ((CHO) 16 vs. (placebo) 35 fold increase in IL-8 mRNA)). Perhaps the greatest evidence for IL-8 expression in skeletal muscle comes from a study by Della Gatta et al. [155] in which IL-8 protein expression was detected in healthy untrained human muscle biopsy specimens at rest and a ∼28 fold increase in IL-8 protein expression was measured 2 h following 3 × 12 sets of unilateral knee extensor exercise in young and old men. This response was not significantly altered by 12 weeks of resistance training. These data provide strong evidence that exercise is a potent stimulus for the muscle cells to produce IL-8. IL-8 release from the muscle into the circulation has only been reported once [203] with only small net releases of IL-8 observed from the muscle. However this release was not large enough to increase systemic levels of IL-8.

However, in comparison to IL-6, the biological role of IL-8 in skeletal muscle is less well understood. As there is little evidence to suggest that muscle derived IL-8 protein is released into the peripheral blood during exercise, it is most likely that it exerts its effects locally. Frydelund-Larsen et al. [205] were able to show that CXC2 receptor (CXCR2) mRNA protein expression can be up-regulated in vascular endothelium and in muscle fibres after concentric exercise (3 h cycling at 60 % $\dot{V}O_2$max) as determined by immunohistochemistry. Combined along with the evidence that exercise up-regulates protein expression of IL-8, this provides support for the notion that exercise induced IL-8 in skeletal muscle acts locally to stimulate angiogenesis through CXCR2 signalling. Therefore, it may be suggested that systemically IL-8 appears to contribute through inflammatory mechanisms, while local skeletal muscle IL-8 appears to be responsible for angiogenesis.

Tumour necrosis factor-α and interleukin-1β

TNF-α plays a number of important roles in inflammation, including activation of leukocytes, and the regulation of the secretion of other pro-inflammatory cytokines [206] such as IL-1β and IFN-γ (cytokines with the ability to induce inflammation). IL-1β is produced by activated macrophages and is important in the initiation and progression of inflammation [207]. The TNF-α and IL-1β response to exercise is equivocal with many investigations finding exercise has little or no effect on circulating levels of either cytokine in the peripheral blood, and it appears that long duration and/or high intensity strenuous exercise is required to induce a large pro-inflammatory, TNF-α, IL-1β blood response. However mixed results have been found following similar types of exercise; Ostrowski et al. [133] found a 2.1 fold increase in plasma IL-1β 1 h following a marathon race in 10 trained male subjects, while Suzuki et al. [208] reported that IL-1β was unchanged in 16 trained male runners following a marathon run and TNF-α was undetectable. Interestingly, IL-1β, IL-4, and TNF-α were unchanged following an iron man triathlon event in 9 male well-trained athletes [209], while IL-1ra, IL-6, IL-10 and heat shock protein 70 were increased markedly, with mild changes in IL-12 and GM-CSF [209]. Following an ultra-endurance running event (200 km), IL-6 was elevated 121 fold accompanied by a 19 fold increase in creatine kinase (CK) but TNF-α again was unchanged [38]. Thirty min of concentric or eccentric bicycle exercise in healthy males was a strong enough stimulus to elevate IL-6, while CK increased only after eccentric exercise and correlated with IL-6, but IL-1β remained under detection levels [143]. Similarly, 12 elite junior handball players completed 4 $\times$ 250 m treadmill sprints at 80 % of maximal personal 100 m speed. IL-6 was increased immediately post-exercise and remained elevated at 1 h post-exercise but IL-1β, its soluble receptor IL-1ra and IL-10 all remained unchanged following exercise [43]. Further, even though a number of pro- (IL-12, IFN-γ,IL-8, MCP-1) including TNF-α, and anti-inflammatory (IL-2R, IL-4, IL-5, IL-7, IL-10, IL-13, INF-α) cytokines were raised following an elite female soccer match, accompanied by elevations in circulating white blood cells, IL-1β was not significantly elevated [145].

It is difficult to ascertain why the same or similar duration or intensity exercise has shown both significant changes and no changes in circulating IL-1β. However, one suggestion could be that due to the small magnitude of positive change noted in those studies that have shown a significant elevation, the inter-subject variability may have a greater effect on mean analysis for these low level cytokines. Further, although it appears that enzyme-linked immunosorbent assay (ELISA) is the predominant method for cytokine analysis, there may be differences in sensitivity and detection limits between ELISA kits from different companies. Also, cytokines are often measured in both serum and plasma, therefore, the component of blood used

for analysis may have an effect on the sensitivity or detection limits of the ELISA kits [210].

Both TNF-α and IL-1β mRNA have been detected in resting human muscle and following acute exercise, where eccentric exercise and possible associations with muscle damage appear to be a greater stimulus for an increase in the pro-inflammatory cytokines [39, 201, 204, 211]. As well, exercise even in trained athletes accustomed to the mode of training can cause elevations in pro-inflammatory cytokine mRNA expression in skeletal muscle [201, 204]. Following 45 min of downhill running (16 % gradient, 70 % max heart rate (HR)) immunohistochemical staining of vastus lateralis muscle revealed a 135 % increase in muscle IL-1 β immediately after exercise and a further increase to 250 % 5 days following exercise. Large accumulation of neutrophils also occurred in muscle and was positively correlated (r= 0.66) with intracellular Z-band damage and IL-1β (0.38) [211]. IL-1β mRNA was also markedly elevated ($\sim$300 fold) in experienced marathon runners completing 3 h of endurance running exercise (70 % maximal oxygen consumption) [204]. To a lesser extent, 2 h of intense resistance training in 30 strength trained men increased TNF-α mRNA $\sim$3 fold and IL-1β $\sim$ 2 fold [201]. In contrast, 60 min of concentric only exercise (bicycle ergometer at 70 % $\dot{V}O_2$peak) did not significantly elevate TNF-α or IL-1β mRNA expression above resting levels [39].

It appears that the pro-inflammatory TNF-α and IL-1β response to exercise is more likely to be localised within skeletal muscle with only very high-intensity or long duration exercise inducing a significant circulating response.

Interleukin-15

The systemic IL-15 response following exercise has not received a lot of attention in current literature but plasma IL-15 was not shown to be up-regulated by endurance exercise (2.5 h treadmill running at 75 % maximal oxygen consumption) [137] but was increased slightly (pre-exercise:$\sim$1.68 vs.post-exercise:1.77 pg/mL) immediately following acute resistance exercise (3 $\times$ 6-10 reps of 13 resistance exercises at 75 % 1-RM) [212]. This response was shown to be unchanged by 10 weeks of resistance training. While there is a paucity of research on the systemic IL-15 response to exercise, research in skeletal muscle tissue supports a greater role for IL-15 following resistance than aerobic exercise.

IL-15 was identified as a muscle-secreted cytokine when it was shown to have anabolic properties via the inhibition of protein degradation and stimulation of protein synthesis in cultured rodent skeletal myotubes that was distinct and independent to that of insulin like growth factor-1 (IGF-1) [213]. Also, human muscle cell cultures exposed to IL-15 were shown to induce an accumulation of myosin

heavy chain (MHC) protein specifically in differentiated myotubes (whereas IGF-I was more effective at stimulating MHC accretion prior to differentiation of myotubes) [214]. Thus, it was suggested that IL-15 may be an anabolic growth factor.

In support, IL-15 mRNA expression was 2 fold higher in triceps muscle compared with soleus muscle, and mRNA expression tended also to be lower in the vastus lateralis when compared with the triceps in human muscle biospy samples [215]. This provides evidence that skeletal muscle IL-15 mRNA is highest in skeletal muscle predominated by type II fibres. However, no differences in IL-15 protein expression were evident between the different muscle types. IL-15 mRNA content also increased 2 fold by 24 h in to recovery following acute resistance exercise (4 × 6-14 reps of leg press and knee extensor exercise with the intention of reaching total exhaustion in each set). Western blot revealed no change in IL-15 protein expression at any of the post-exercise time points (6, 8, 24 or 48 h) [215].

Similar to the lack of change in plasma IL-15 following aerobic exercise [182], skeletal muscle IL-15 mRNA expression was detected at rest, but was not elevated immediately following a 3 h run ($\sim$70 % $\dot{V}O_2$max) in trained marathon runners [204] but neither was it elevated following 2 h of resistance exercise (10 exercises, 4 sets each, 10 reps with 2-3 min rest intervals) in strength trained athletes [201]. Based on the results from Nielsen et al. [215] IL-15 mRNA may be up-regulated closer to 24 post-exercise and as such an immediately post-exercise sample is unlikely to reveal changes in mRNA expression for this particular cytokine.

Current research suggests that IL-15 may be an important mediator of muscle anabolism responses to resistance exercise in humans. However, more research in athletic populations is required to improve understanding of this area and confirm the role of IL-15 in exercise induced adaptation to resistance exercise.

Interleukin-7

Investigations into the IL-7 systemic response to exercise are restricted to a single study to the author's knowledge. Immediately following an elite female soccer match IL-7 was significantly elevated above pre-exercise concentrations. Seventy two hours after the first game was played a second elite match was played and IL-7 concentration was not elevated significantly from pre game 1 levels which could not be explained by CHO availability, exercise intensity or the repeated bout effect [145].

Similarly, only one study has investigated the presence of IL-7 mRNA in human skeletal muscle [216]. Resting muscle biopsies from the vastus lateralis and trapezius were taken after 2 weeks and 11 weeks of a strength training programme (3 × per week with each workout consisting of 1-3 sets at 7-10 RM of leg press, leg extension, leg curl, seated chest press, seated rowing, lattisimus pull down, bicep

curl, and shoulder press). IL-7 mRNA increased by 3 fold in vastus lateralis and 4 fold in trapezius after 11 weeks of strength training. A concurrent *in vitro* study allowed the authors to confirm that IL-7 is produced and secreted by differentiated muscle cells and that IL-7 was able to affect myogenesis and migration. [216].

More specific research during and following exercise needs to be undertaken before it is possible to make conclusions about the specific role of exercise induced IL-7. However, it may have a role to play in muscle cell development.

Interleukin-4 and interleukin-2

Within the construct of this review, circulating IL-4 and IL-2 concentrations following exercise are not as well investigated as some of the other cytokines, but they have been measured following varying intensities of endurance exercise. Generally small increases in IL-2 and IL-4 are seen following exercise above 65 % $\dot{V}O_2$max [145, 217–219] but Chen et al. [220] reported a small but significant decrease in IL-2 following a 21 km run and Boghrabad et al. [221] recorded a significant decrease in plasma IL-2 (and IL-6, IL-1ra and IL-1β) after 3 months of an aerobic training programme in untrained individuals. Other studies report no change in IL-4 following 60 min of treadmill running at different intensities and a marathon race [208,222]. IL-2 response to exercise may differ depending on training status, with trained runners showing a 50 % decrease in IL-2 following a 5 km running race [223], while no changes in IL-2 were observed following 60 min of cycling exercise at 75 % $\dot{V}O_2$max [224]. In resistance trained men, IL-2 was significantly decreased from baseline at 15 min post high force (greater load) squat exercise, but not following high power (lower load, faster lifting velocity) [225] suggesting that the acute stress of high force versus high power workouts induce differential effects on circulating IL-2.

IL-2, a pro-inflammatory cytokine is multi-functional in the inflammatory response, including proliferation and differentiation of T-cells [226] and the stimulation of white blood cells on the endothelial surface of skeletal muscle (demonstrated in rats) [227]. On the other hand IL-4 is an anti-inflammatory cytokine which can be produced by neutrophils [228], although skeletal muscle has been shown to express IL-4 protein where it is thought to stimulate myogenesis [229]. Prokopchuk et al. [230] and Della Gatta et al. [155] both detected IL-4 protein in skeletal muscle at rest and both demonstrated training effects. Prokopchuk et al. [230] demonstrated a mild decrease in IL-4 protein at rest following 6 weeks of maximal strength training, but a mild increase in IL-4 protein resting expression after combined maximal strength and ballistic training. Della Gatta et al. reported a 1.4 fold decrease in IL-4 protein 2 h following acute knee extension exercise and a 1.7 fold increase in

IL-4 protein after a 12 weeks training period [155]. The significance of these exercise induced alterations in IL-2 and IL-4 are yet to be elucidated.

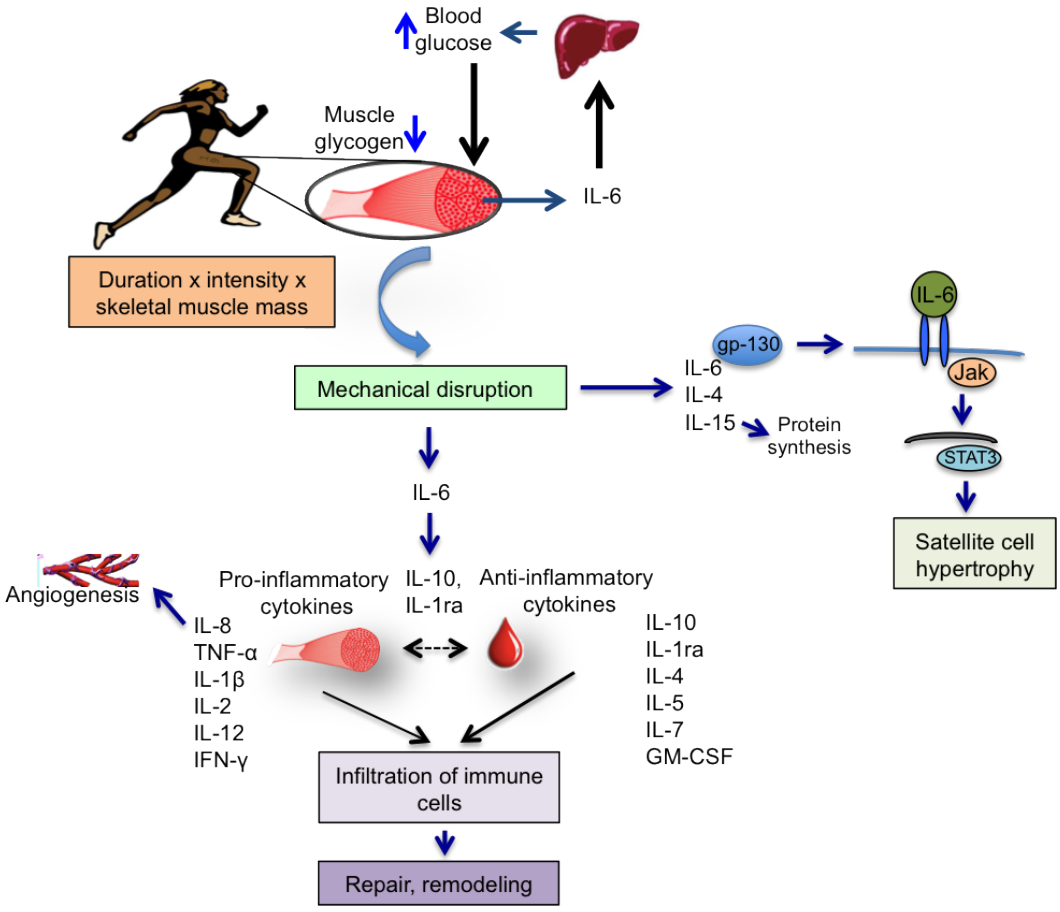


Figure 3.2: IL-6 initiates a pro- and anti-inflammatory cytokine response in the peripheral blood and local skeletal muscle that provokes the infiltration of immune cells for repair and remodelling in response to mechanical disruption. This response is dependent on the exercise intensity, duration, contraction type and skeletal muscle mass involved. IL-6, IL-4, IL-15 promote muscle hypertrophy; IL-6 through Jak/STAT signalling and myogenesis. Skeletal muscle produces and releases IL-6 in response to contraction and lowering glycogen stores, IL-6 initiates cross-talk with the liver to increase glycogenolysis and increase blood glucose levels. IL-8 stimulates angiogenesis within the endothelial microvasculature.

Cytokines and training adaptation

Exercise training is associated with a number of physical and physiological adaptations including changes in body composition resulting in lowered fat mass [231] and increases in skeletal muscle mass through muscle hypertrophy [232]. As well, training increases skeletal muscle resting glycogen stores [128, 129], a greater capacity for beta-oxidation and induces an increased ability to rely on fat as a fuel

source [233, 234]. Reliance on blood glucose and skeletal muscle glycogen as a fuel source during exercise is reduced [235]. As a consequence, athletes can lift heavier loads, and run or cycle for longer for the same absolute and/or relative intensity. There are only a small number of training studies involving plasma and muscle biopsy samples in healthy trained and untrained humans, and evidence is equivocal as to whether training has an effect on basal or exercise-induced systemic and local cytokine responses.

Systemic responses

At present, evidence is limited as to whether exercise training and the associated physical and physiological changes significantly modulate either basal levels or the acute cytokine response to exercise in healthy humans. Of the small number of studies that have investigated the effects of either training status or exercise training on systemic inflammatory cytokines, only one reports a lowered basal IL-6 concentration [236]. Elite competition skiers completed an incremental treadmill test to exhaustion during the competitive season (high training load) and off season (low training load). IL-6 was only increased ∼2 fold following the treadmill test irrespective of season and while there appeared to be an elevated IL-6 response in the 2 h recovery period during the off season this was not significant. Basal IL-6 levels were mildly lower during the recovery season [236]. In contrast, young physically inactive males were assigned to a 3 × per week for 12 weeks concurrent endurance and resistance training intervention (20 min treadmill exercise at 70-80 % HR reserve and 2 × 8 reps at 70-80 % 1-RM of 8 resistance exercises). Only resting blood samples were taken for this study and IL-6, IL-1β and TNF-α were analysed pre and post the training period. There were no significant training effects on basal IL-6, IL-1β or TNF-a [237].

In highly trained male rowers who completed a 6000 m rowing ergometer performance test 1 year apart, no differences in basal levels of IL-6 or TNF-α were observed [238]. Acute exercise increased IL-6 significantly above pre-exercise levels at 0 and 30 min, however interestingly, post-exercise IL-6 response was higher after 1 year training. TNF-α was only increased after test 2 when compared to pre-test values. The higher values of post-exercise IL-6 and TNF-α corresponded with increases in rowing performance [238], however it is difficult to suggest a mechanistic reason for this response and more research investigating the long-term training effects on these responses may reveal some important and interesting roles for cytokines in exercise induced adaptation. In contrast, 24 high level endurance cyclists completed 8 weeks of progressive endurance training and were then split in to 2 groups. One group continued with the same progressive training, while the other completed 3 weeks of tapering (50 % drop in training load). A 40 km cycling time trial (TT)

was completed at week 1, 4, 8, 9 and 11 and post-exercise blood samples were measured for IL-6, IL-1β and TNF-α. The 40 km TT was faster at 9 and 11 weeks in the taper group, which coincided with a lower IL-6 concentration in the taper group at 9 and 11 weeks, and IL-1β which was lower at 11 weeks in the taper group compared with the progressive exercise group. However, this study did not measure resting cytokine level at any time throughout the study so it is difficult to ascertain whether the lowered post-exercise cytokine results could be due to lowered basal concentrations, rather than a lowered acute response to the exercise test [239].

Following acute HIT exercise (4 intervals at 60 s on, 75 s active recovery at 100 % $\dot{V}O_2$max preceded by a graded exercise test and 20 min passive rest) in recreationally active males, serum IL-6 was significantly elevated above resting levels immediately post, as well as 15, 30 and 45 min post-exercise [200]. To a lesser extent IL-8 was elevated from 30 min post-exercise while mild increases were seen in TNF-α and MCP-1 and an increase in IL-10 at 45 min, with no changes in IL-1β, GM-CSF, IFN-γ were seen. Two weeks of strenuous HIT training was undertaken (6 HIT sessions: 8-12 intervals) and the cytokine response to the acute interval session was not attenuated. To account for the absolute increases in strength following a period of 7 weeks of resistance training (45-60 min 2 $\times$ week), plasma cytokine responses were measured after an acute resistance trial (5 $\times$ 10 RM leg press) at pre-exercise, and following the same acute trial with the same absolute load (kg) and same relative load (%) after 7 weeks training [240]. IL-1β concentrations were higher during exercise and recovery in both the absolute and relative trials compared with pre-exercise, where as IL-6 and IL-10 were only elevated following the relative load test suggesting that exercise intensity plays an important role in the cytokine response to exercise. IL-1rα response was also higher after training which may have counter-acted the effects of elevated IL-1β [240].

Overall, it appears that studies investigating the effect of training on both the resting and acute exercise responses of systemic inflammatory cytokines have found equivocal results. Furthermore, as the results are inconsistent it is difficult to allude to mechanisms that may explain the training induced adaptation or lack thereof in systemic inflammatory cytokines.

Local skeletal muscle responses

To date, the only studies investigating the effect of training on cytokine expression in skeletal muscle in healthy humans have been undertaken following endurance or resistance exercise and knee extensor activity [147, 155, 156, 230, 240, 241]. Resting cytokine mRNA expression of IL-4, IL-13, IL-4ra and IL-13ra and IL-4 protein expression were measured in biopsy samples taken 3 days before and 7 days after

6 weeks of training in 24 male physical education students [230]. Students were split into a traditional maximum strength training group (3 × week 5 sets of 3-RM bench press) or a combination of maximum strength and ballistic stretch shortening contractions (day 1:3-RM bench press, day 2: bench throw at 30 % 1-RM and day 3: 10 × ballistic push ups). Training increased the resting mRNA expression for both IL-4 and IL-13 and their receptors, and the percentage increase was significantly greater in the max strength training group for all but IL-13ra despite the similar increases in bench press 1-RM between groups. IL-4 protein expression was slightly decreased following training in the maximum strength group and slightly increased in the combination group however these changes were not significant [230]. This study demonstrates that, like 1L-15, IL-4 and IL-13 may have a possible role in skeletal muscle remodelling responses to strength training which is likely more pronounced with maximum loads.

Further, mRNA expression of IL-6 at rest and post acute exercise were quantified from the femoral artery prior to and following 10 weeks of knee extensor exercise (1 h × 5 per week of knee extensor exercise on a modified Krogh ergometer at 75 % maximal velocity) in 7 untrained participants [147]. Before and after the training period, participants completed knee extensor exercise for 3 h at 50 % of maximal velocity, IL-6 mRNA was measured at rest, immediately post-exercise and 2 h in to recovery. IL-6 mRNA content was increased by acute exercise in skeletal muscle by 76-fold before training and only 8-fold after training despite a higher absolute work load (due to adaptive responses to habitual exercise) post training. Pre-exercise muscle glycogen content was increased ∼74 % following training but decreased to the same extent as pre training following acute exercise and muscle glycogen content correlated negatively with IL-6 mRNA (r= 0.34). Plasma IL-6 was increased up to ∼5 pg/mL immediately post-exercise and 2 h in to recovery but this response was unaltered by training. Interestingly, following the same acute and training protocol as the above study in similar participants, muscular mRNA expression of the IL-6 receptor (IL-6R) at rest was significantly increased after training (∼100 %), while the same absolute values of IL-6R mRNA were achieved immediately post and 2 h post-exercise. IL-6-mRNA expression was also unaffected by intramuscular glycogen levels [241] and plasma IL-6R was unaltered which suggests a predominant local role for the IL-6R during exercise. Together these studies convey that IL-6 mRNA was down-regulated locally following training due to a lowered dependence on extra-muscular energy substrates or that enhanced receptor expression increases the sensitivity to IL-6 and a possible down-regulation of IL-6 during exercise. It has also been demonstrated that IL-6 alone did not stimulate glucose transport in mouse soleus muscle, but upon the introduction of the soluble IL-6R glucose transport was directly stimulated [242]. Preliminary work suggests an important role of the IL-6R particularly within the skeletal muscle and further research in this area is warranted.

In young healthy males, protein expression of IL-4, IL-6, IL-8, IL-10, IL-13 and TNF-α were measured in muscle homogenates at 2 h in to recovery [155] following unilateral knee extensor exercise on a dynamometer (3 $\times$ 12 reps at maximum with 2 min rest). TNF-a was not detectable in 23 of 28 samples, while following acute exercise IL-6 was increased $\sim$4 fold, MCP-1 increased $\sim$16 and IL-8 increased a considerable $\sim$28 fold, while a small but significant increase was seen in IL-10 pre-training. IL-4 was found to be decreased $\sim$1.4 fold. Participants then completed 12 weeks of resistance training (2 $\times$ 8-12 reps at 50-80 % 1-RM for 6 upper and lower body resistance exercise). Cytokine expression at rest was unchanged with training, further the increases in IL-6, IL-8, MCP-1 and IL-10 protein expression were similar following acute resistance exercise pre and post 12 weeks of training, while IL-4 was increased $\sim$1.7 fold with the authors suggesting that this may reflect an adaptation to support myoblast fusion and subsequent myogenesis. Significant increases in leg extension, leg press and bench press estimated 1-RM performance were achieved after the training period. The pre and post training exercise protocols in which the muscle biopsies were taken consisted of maximal knee extensor exercise. Therefore, rather than the post trial being completed at a lower relative intensity due to training adaptation the pre and post testing was completed at the same relative intensity. As such, it is therefore unclear whether the cytokine response would have been attenuated post training if exercise was performed at the same pre-exercise training load and subsequently a lower exercise intensity.

Following the same exercise protocol as the above study in 13 healthy untrained men, IL-6 protein expression was increased in skeletal muscle by $\sim$ 17.5 fold at 3 h post acute exercise before training and $\sim$5 fold after training, however these fold changes were not significantly different [156]. Similarly to the above study, resting IL-6 protein expression was unaltered by training. It is difficult to suggest why the fold change in IL-6 protein expression following acute exercise is higher in this study than the latter but it could be due to the differences in timing of the biopsies (2 h post-exercise [155] versus 3 h post-exercise [156]) or possibly due to individual variation in the exercise induced responses which may be heightened due to the untrained status of the participants.

Similar to the systemic cytokine responses to exercise training, the role of training on skeletal muscle cytokine response to exercise is unclear. Conclusions are difficult to draw based on the small number of studies that have investigated the role of training on the acute local cytokine response to exercise, however it does appear that these responses may not be easily attenuated by training. Further investigations are required to confirm whether training of particular exercise modes of specific intensities and durations can modify local cytokine responses in order to improve our understanding of the role of these cytokines following exercise.

To summarise, few studies have investigated the effect of a period of exercise training on the resting local or systemic cytokine expression in a healthy population. Fewer studies have investigated the effect of training on the acute local and/or systemic cytokine response to exercise. Due to differences in exercise protocols, mode of exercise, intensity of exercise and differences in the methodology for measuring cytokines (real time PCr, bead based multiplex assay, ELISA) it is difficult to determine consistent conclusions about the effect of a period of training on cytokine expression. However, from the information gained from the previous studies, it appears that controlled and structured endurance or resistance exercise training for a period of 2 - 12 weeks does not significantly attenuate resting or acute exercise cytokine responses in skeletal muscle or within the peripheral blood. However, further studies of greater training intensity, or longer training duration that allow for further physiological adaptations may yet demonstrate exercise induced alterations in cytokine concentrations. Without evidence however, that IL-6 plasma or skeletal muscle IL-6 can be altered by training in healthy humans. Particularly when alterations are observed in the plasma glucose and skeletal muscle glycogen content after training, the role of IL-6 as a mediator of exercise glucose metabolism may be questioned. As well, if cytokines are unaltered or modified by training or training status it may suggest that the acute cytokine response to a particular exercise protocol is an essential constituent of exercise induced repair and adaptation.

Sex differences in the exercise induced cytokine response

Currently, only a limited number of studies have compared the exercise induced cytokine response between males and females, with IL-6 the predominant investigated cytokine.

Plasma concentrations of IL-6, IL-10, IL-1ra and IL-8 were elevated immediately following a competitive marathon race in trained runners (12 females, 84 males) and remained above pre-exercise levels at 1.5 h in to recovery [196]. The pattern of change in plasma cytokines was not different between female and male runners. Twelve male and female recreationally active students completed a submaximal (4 min incremental step test, 4 min each step plus 25 min cycling at 55 % of peak power output) and maximal cycle ergometer (stepwise incremental cycle test until exhaustion followed by cycling at 55 % of peak power output up to 45 min of total exercise) exercise trial [243]. Plasma IL-6 was not different between males and females at rest nor during the submaximal trial. IL-6 was increased after both exercise trials, immediately post-exercise and 30 min post. However, at 60 min post maximal exercise, the female IL-6 values continued to rise while the male

values dropped back towards baseline values [243]. In contrast, significant differences in resting plasma levels of IL-6 and TNF-α were observed in elite male and female handball players [244]. Following resistance exercise (3 $\times$ 10 reps of upper and lower body resistance exercises at 60 % 1-RM) there was a modest but non-significant increase in TNF-α and a significant increase in IL-6 at 2 h post-exercise, with the increase in IL-6 being significantly greater in men compared to the increase in women [244]. Helge et al. [245] reported no differences in IL-6 release across the arm or leg during whole body exercise (90 min arm and leg ergometer exercise at 60 % $\dot{V}O_2$max) between males and females. The differences in results in these studies suggest that gender differences in the cytokine response to exercise may be dependent on the mode and/or intensity of exercise. Menstrual cycle phase and hormonal contraceptive methods were not controlled for in any of these studies. This may be important as Timmons et al. [246] provided evidence that oral contraceptive use may affect exercise induced plasma IL-6 response.

In contrast to the previous studies, Timmons et al. [246] did control for menstrual phase (follicular) and contraception (triphasic oral contraceptive (OC)). There was no difference in the resting and exercise induced IL-6 response to 90 min of cycling (65 % $\dot{V}O_2$max) between males and females, but there was a trend ($p = 0.06$) for increased IL-6 between female OC users and non OC users exercising in the follicular phase. This suggests that OC use may have effects on the kinetics of cytokine response to exercise in females, and until further research is conducted to determine the significance of this effect, OC use should be monitored and controlled for in future research investigations. A female only study, investigated the effect of the follicular and luteal phases of the menstrual cycle on exercise induced IL-6 following 75 min of HIT training (50-110 % of v$\dot{V}O_2$peak for 1-2 mins at a time). Significant increases in IL-6 after exercise were recorded but there were no effects for menstrual phase, further IL-6 was not related to delayed onset muscle soreness [247]. These studies suggest that OC use but not menstrual phase may alter the IL-6 response to exercise.

To date, research into specific sex differences in the cytokine response to exercise are limited. Also, not all studies using female participants control for menstrual cycle nor hormonal contraceptive methods in females and this is important as there may be subtle differences in the exercise-induced cytokine response to exercise during these varying conditions. A greater body of research needs to be undertaken to ascertain whether true sex differences exist in the cytokine response to exercise, which must include the control of menstrual phase and hormonal contraceptives, as well encompassing all modes of exercise and a greater array of cytokines. Further, sex differences in IL-6 concentration following exercise should be differentiated between metabolic and inflammatory responses.

Summary

Exercise is a potent stimulus for the production and circulation of inflammatory cytokines. IL-6, the immuno-modulator protein is the predominant systemic cytokine seen following exercise of any mode, but can be attenuated upon the ingestion of CHO during endurance but not heavy eccentric exercise. While circulating IL-6 levels and net release from skeletal muscle appear to be modified by CHO ingestion or skeletal muscle glycogen status, IL-6 mRNA expression in skeletal muscle appears to remain unaltered. Particularly during exercise with a large eccentric component resulting in adjustments of skeletal muscle architecture, a local pro-inflammatory environment (IL-8, TNF-α, IL-1β) is triggered within the skeletal muscle, while predominantly anti-inflammatory cytokines are elevated in the peripheral blood to respond to and attenuate the local inflammation. Further, the release of pro-inflammatory cytokines from the local skeletal muscle in to the blood stream have not been reported and thus may account for low blood levels of IL-1β and TNF-α following exercise. Thus it appears that the pro-and anti-inflammatory cytokine response is balanced between the local pro-inflammatory response and the systemic anti-inflammatory responses. The effect of training status and chronic training on the resting or post-exercise cytokine concentrations remain controversial due to the small body of investigative studies. However, present evidence suggests that the cytokine response to exercise is not easily altered by training in healthy populations and may therefore be a vital component of the restorative and adaptive processes to exercise. True sex differences in cytokine responses are still to be fully investigated, particularly following resistance and high-intensity exercise. However, it is important to control for menstrual status and hormonal contraception in females until effects of these on cytokine responses are better understood. The exact contributions of cytokines to exercise induced muscle damage, mobilisation of circulating immune cells, and metabolism remain to be fully elucidated.

Epilogue

Monitoring training load in team-sport athletes: a role for inflammatory cytokines?

Appropriately structured training programmes are designed to improve or maintain performance during pre-competition and competition phases. However a challenge for sport science practitioners is the monitoring of training load via acute alterations in markers of external and internal stress in order to determine that training is balanced to stimulate over-load and training adaptation while preventing over-

training and maladaptation. While external training loads are easily quantifiable from the measurement of physical work by recording exercise duration, speed, power and load using such innovations as GPS technologies, accelerometers and power meters [248–252], monitoring of internal training load has proven to be more difficult.

Heart rate (HR) and ratings of perceived exertion (RPE) are two of the most established measures of measuring training stress [253], having been used to monitor load during resistance [254, 255], endurance [256, 257], intermittent and team-sport exercise [258–260]. HR is a popular indirect method to estimate acute training load due to its ease of measurement and relationship with $\dot{V}O_2$ and energy expenditure [261, 262], however there is little consensus on how best to interpret HR data in order to quantify the internal load, particularly during intermittent exercise. While TRIMP scores [263] have been developed to extend the ability to use HR as a measure of training load via the multiplication with exercise intensity and duration, its use in non-aerobic steady state exercise has limitations [264]. Further, the use of HR zones have been shown to be invalid for describing training intensity in soccer players [265].

RPE is a well-utilised measure of an athletes subjective assessment of the physiological strain/response to exercise. However limitations exist with RPE in that during lower intensity exercise RPE is dominated by peripheral sensations of fatigue, while during higher-intensities of exercise central mechanisms/sensations such as HR and increasing blood lactate concentrations may dominate the subjective measure of training stress [45]. Further, while HR and RPE have a high correlation during endurance exercise [266], this relationship is poor in intermittent team-sport based exercise [258]. Despite this however, RPE was found to be a valid marker of exercise intensity across a number of different intensity based soccer drills [258]. RPE was also found to be effective at distinguishing differences in perceived exertion between different intensities of back squat exercise but RPE was not directly related to the loading that was used [254], thus suggesting that RPE may be a valid measure of subjective internal training load within modes of exercise, but may have limitations in the ability to subjectively compare internal training stress between divergent modes of exercise that differ in their predominant base of fatigue (peripheral vs. central). This may be a limitation specific for team-sport athletes who complete multi-dimensional training programmes due to the requirements of their matched-based competition.

Therefore, a quantitative measure of internal training stress that is representative of training intensity, duration and skeletal muscle mass involvement may provide an accurate and functional understanding of the physiological cost of exercise and tolerance of an athlete across divergent modes of exercise and may provide a sensitive measure for athletes involved in intermittent team-sport exercise. Ac-

curate feedback regarding the physiological response of training loads may assist in the subsequent alterations of training prescription, while long-term monitoring of exercise tolerance may enhance the training process and performance improvement.

Serum creatine kinase (CK) has a been a popular bio-marker for training stress due to the relative ease of measurement however there is high variability in measurement [267] and its relationship to muscle damage is not always directly apparent [268]. Salivary and serum cortisol and testosterone measurements have also previously been used as an indicator of the anabolic/catabolic environment [269–271], however recent debate over the role of acute alterations in endocrine hormones in stimulating skeletal muscle protein synthesis [104, 105, 107, 108] makes the interpretation of the testosterone/cortisol ratio difficult.

As inflammatory cytokines are known to be elevated in an intensity, duration and skeletal muscle mass dependent manner [31, 32] during exercise they may be an appropriately sensitive bio-marker for the measurement of acute and chronic internal training stress/load across divergent modes of exercise that are not easily comparable with simple measures such as HR and RPE. Further, due to the proposed role of inflammatory cytokines in skeletal muscle repair and remodelling through inflammatory mechanisms [133], hypertrophy [175, 176, 179, 214] and angiogenesis [193, 194], as well as a role in skeletal muscle - liver cross-talk and carbohydrate metabolism [33, 159, 160], these bio-markers may provide greater insight between systemic and local (skeletal muscle) responses and a more precise insight in to the internal physical load of an athlete. While systemic inflammatory cytokine responses have previously been measured following competitive soccer match performance in males [272] and females [145], their measurement following acute and chronic concurrent repeated-sprint and resistance exercise designed around the requirements of team-sport athletes has not yet been quantified in the literature. Therefore while there is theoretical support for the use of inflammatory cytokines as a marker of internal training stress, evidence of their use in practise is currently limited.

CHAPTER 4

The inflammatory and hormonal responses to single-mode repeated-sprint and resistance exercise in female team-sport athletes

Abstract

This study evaluated the effect of short duration repeated-sprint and lower body resistance exercise on circulating inflammatory cytokine and endocrine hormones. Eight trained female team-sport players (18-28 years) participated in the study. Participants completed 2 trials separated by 7 days; 1) Resistance trial (RT): 6 × 6 sets of back squat exercise at 80 % 1RM, sets separated by 3 min rest 2) Repeated-sprint trial (RSA): 4 × 6 sets of 20 m maximal shuttle sprints, sets separated by 3 min rest. Venous blood samples were collected at pre-exercise, immediately post-exercise (0 min) and 15 min, 30 min, 60 min, 120 min and 840 min during recovery. Exercise of both modes increased the inflammatory cytokine Interleukin (IL)-6, above pre-exercise levels at 120 min in to recovery (pre-ex: 3.31 ± 2.11-5.85 vs. 120 min post-ex: 7.05 ± 3.96 - 9.04 pg/mL, $p < 0.05$). Cortisol and growth hormone concentrations were lower than pre-exercise levels by 60 and 120 min post exercise respectively ($p < 0.05$) in both exercise trials. Area under the curve (AUC) for cortisol showed a strong trend ($p = 0.052$), to differ between exercise trials with higher values following RSA compared with RT (RSA: 697.17 ± 206.92 vs RT: 515.23 ± 171.89 ng·min·mL^{-1}). Cortisol was significantly lower at 840 min in to recovery following RSA compared with RT ($p < 0.05$). Significant differences in the detectable values of post-exercise inflammatory cytokines in plasma and serum were also observed. The increase in IL-6 following exercise may be indicative of a role in muscle

repair. In conclusion, the training stress was well tolerated by the athletes and similarly tolerated between exercise modes. A larger training volume may be required to cause significant stimulus of the endocrine and inflammatory systems in trained female athletes.

Introduction

Repeated-sprint ability training (RSA) and lower-body resistance exercise (RT) are commonly used in sports that require repeated aerobic and anaerobic type efforts, such as soccer [30,118]. Sprint training has been shown to cause large ion disturbance [273], accumulation of intramuscular lactate [274], and a molecular profile closely associated with mitochondrial biogenesis [275]. In contrast, RT exercise is associated with anabolism and increases in protein synthesis [276]. Therefore, it would be expected that the systemic physiological response to exercise may differ between these two modes of exercise. RSA is considered to be a crucial fitness component that can predict match play performance [121] while leg strength is correlated with sprint speed [277] and aids in kicking and tackling performance in sports. Therefore, team-sport players should include both of these exercise modes in a training programme. Consequently it may be important to understand the inflammatory and hormonal consequences to these individual and divergent modes of exercise in an effort to gauge the intensity of exercise by way of internal training stress and subsequently the ability of the athletes to tolerate the exercise in order to adequately and effectively structure training programmes. Analysis of circulating factors following exercise may be a manageable way of determining the physiological cost of exercise.

Recent research demonstrates that some exercise modes such as sprint interval training, concurrent strength and endurance training and soccer match-play can lead to both, a change in the components of the growth hormone↔insulin-like growth factor-1 (GH↔IGF-1) axis [43,98,278], as well as an increase in circulating cytokines such as interleukin (IL)-6, IL-10, and tumour necrosis factor-alpha (TNF-α) [43, 145]. The evaluation of this circulating systemic response of the endocrine and immune/inflammatory systems to exercise may help to objectively quantify exercise intensity and internal training stress, which could aid training design. IL-6 may also be used as an indicator of the metabolic stress during exercise as circulating IL-6 levels during exercise have been shown to be influenced by muscle glycogen stores [279]. To date, no studies have simultaneously studied the systemic inflammatory and hormonal response patterns following RSA and RT exercise of similar total work volume in the same trained individuals.

Importantly, serum versus plasma profiles for inflammatory cytokines have not been evaluated in exercised human samples. While both plasma and serum

are derived from whole blood, variation in biochemical processing mean subtle differences exist between the components of plasma (contains fibrinogen) and serum (does not contain fibrinogen). Whether differences in these matrices affect the detectable levels of inflammatory cytokines following exercise when using multiplex assays is currently unknown, and it may be useful to investigate to determine the comparability of studies measuring post-exercise cytokine responses in plasma or serum.

Therefore, the aim of the current study was to determine the inflammatory and hormonal response profile to short duration single-mode RSA and RT exercise. A secondary aim was to determine if there were differences in the detectable levels of inflammatory cytokines between plasma and serum post-exercise samples.

Methods

Overview of experimental protocol

Participants reported to the laboratory 1 week prior to the first experimental trial to complete a 1-repetition maximum (1-RM) test for the back squat exercise, which was used to calculate the individual load to be lifted during the RT trial. A familiarisation of the RT and RSA protocols were also conducted. Participants completed 50 % of each protocol so as to understand the procedures involved with each exercise mode and ensure maximal effort during experimental trials. Participants also completed the Yo Yo intermittent recovery test (YYIRT1) in the week prior to the first experimental trial as a measure of aerobic fitness. Each participant completed 2 experimental exercise trials 1) Resistance trial (RT) 2) Repeated-sprint trial (RSA), in a randomised, cross-over design. Exercise trials were performed in the evening between the hours of 17:30-18:30 h to coincide with normal team-sport training hours, which were preceded by a standardised meal and a 4 h fast. An indwelling venous cannula was inserted 10 min prior to the pre-exercise blood draw. Subsequent blood draws were taken immediately post-exercise (0 min) and 15 min, 30 min, 60 min, 120 min and 840 min during recovery (see Figure 4.1).

Participants

Eight female team-sport players (soccer, hockey, netball, touch rugby) [mean (standard deviation (SD)): age 21.9 (3.0) y; body mass 71.8 (7.0) kg; $\dot{V}O_2$max 45.0 (3.5) mL·kg^{-1}·min^{-1}; 1-RM 78.8 (10.8) kg] who had some lower body resistance training experience in the past but were not currently resistance training volunteered to participate in this study within season. Exclusion criteria included the use of

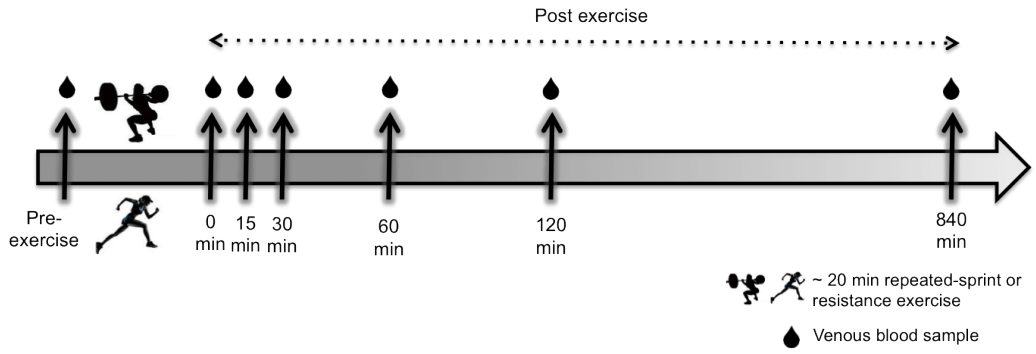


Figure 4.1: Overview of experimental protocol.

anti-inflammatory medication or medication with known effects on circulating hormones and cytokines. Following the explanation of the purpose and aims of the current study, informed written consent was obtained from all participants. All experimental procedures were approved by the Auckland University of Technology Ethics Committee.

Participant controls

Menstrual and contraceptive controls

Due to possible effects of the menstrual cycle and/or oral contraceptive use on circulating levels of inflammatory cytokines [246] and endocrine hormones [280] and performance [281, 282], the following contraceptive control [283] was applied. Participants were required to be taking a monophasic combined oral contraceptive pill (OC: Levonorgestrel 0.15 mg and Ethinyloestradiol 0.03 mg) and were also required to have had a regular menstrual cycle prior to OC use. Participants were instructed to remain on the active hormone tablets of their contraceptive pills for the full duration of the study.

Dietary and exercise controls

Participants were required not to perform any exercise 24 h prior to the experimental trials. Participants were also asked to keep a 48 h food diary inclusive of the day prior to the 1st experimental trial and the day of the experimental trial including post trial dinner (eaten between 21:00-22:00 h) and were asked to maintain the same diet prior to the second experimental trial. Participants were provided with a standardised meal ($\sim$ 51 KJ energy per kg of body mass; 78 % carbohydrate, 8 % Fat, 14 % Protein) to be eaten 4 h (13:00 h) prior to experimental trials and water was able to be consumed ad libitum pre, during and post-exercise. Following dinner on the night of the experimental trials, participants were asked to refrain from

eating or exercising again until after the 840 min blood sample. Participants were not permitted to consume caffeine or alcohol within 24 h of experimental trials. Put text in red. Participants were instructed that recovery from exercise should consist of passive rest only and that no other recovery modalities were to be used (i.e. the use of ice, massage or anti-inflammatory products).

Preliminary testing

One-repetition maximum back squat test

Participants underwent a 1-RM test for the back squat exercise to determine the subsequent load to be lifted during the experimental trial (80 % of 1-RM). Following warm up sets at 50 % and 85 % of estimated 1-RM a maximum of 5 1-repetition trials were used to determine the 1-RM as outlined by Vingren et al. [284]. A lift was considered successful once participants had descended to the point where the tops of the quads were parallel with the floor. The reliability of this test in our laboratory is high, intraclass correlation coefficient (ICC) = 0.97 and coefficient of variation (CV) = 2.5 %.

Yo Yo intermittent recovery test level 1 (YYIRT)

The level 1 version of the YYIRT was completed following the protocol previously described by Krustrup et al. [285] Heart rate (HR) during the YYIRT test was measured with a RS800CX Run polar HR monitor (Polar, Kajaani, Finland) and maximum HR achieved during the YYIRT test was recorded. Distance covered during the YYIRT test was converted to a $\dot{V}O_2$max score [286]. Female team-sport players that achieved an estimated $\dot{V}O_2$max score above 40 $mL \cdot kg^{-1} \cdot min^{-1}$ were included in the current research.

Experimental procedure

The specific resistance and repeated-sprint protocols described in detail below were designed based on the specific performance requirements of field-based team-sport athletes. Both protocols were designed to provide approximately the same volume of exercise activity and similar work:rest ratio to allow the comparison of internal training stress between two divergent modes of exercise with similar external loading which are difficult to compare with traditional internal load parameters such as HR and ratings of perceive exertion (RPE).

Resistance exercise protocol

Given the relationship between maximal squat performance and short sprinting and jumping ability in team-sport athletes [14–16, 123] it is likely that a training programme with squat exercises as a major component would result in improved team-sport performance. Therefore, the following squat protocol was designed and implemented.

Prior to commencing the experimental resistance session, participants were required to complete a standardised warm-up consisting of 1 set of 6 repetitions with a 20 kg Olympic weight lifting bar followed by 1 set of 6 repetitions at ∼50 % of their experimental load, followed by a further set of 6 repetitions at ∼70 % of their experimental load.

The RT protocol consisted of 6 sets of 6 repetitions of back squat exercise with 80 % of 1-RM with 3 min of passive rest between sets for a total of ∼6 min exercise activity and total duration (including rest periods) of ∼21 min.

Repeated-sprint protocol

An RSA protocol was designed that included shuttle sprints similar in duration and length to sprints completed during team-sport match-play [7, 9, 116, 117] but at a greater frequency in order to cause overload.

Before commencing the experimental sprint protocol, participants were required to complete a standardised warm-up protocol. Participants were asked to jog 6 lengths of a 20 m shuttle at a self-selected pace. This was followed by 6 progressive sprints (2 sprints at ∼60 % maximal speed, 2 sprints ∼70 % maximal speed and 2 sprints at ∼80 % maximal speed).

The RSA protocol consisted of 4 sets of 6 × 20 m maximal running shuttle sprints. Participants were instructed to begin from a standing start 30 cm behind the start line. When instructed to go, participants were told to sprint maximally towards a line 10 m away, placing both feet over the line, participants were then required to turn and sprint maximally back to the start line. Participants completed 1 sprint every 20 s for a total of 6 sprints in each set. Sets were separated by 3 min of passive rest. Total exercise activity was ∼8 min and total duration (including rest periods) of ∼17 min.

Heart rate and ratings of perceived exertion

HR and RPE were recorded throughout the RSA and RT exercise protocol to determine the physiological effort associated with each mode of exercise. HR was

measured with an RS800CX Run polar HR monitor (Polar, Kajaani, Finland) at rest and post each sprint set or squat set. RPE was recorded with use of the 6-20 point Borg scale [287] following each RSA and RT set to gain a subjective measure of how difficult each participant found the exercise protocol.

Blood collection and analysis

Blood samples were drawn from an indwelling cannula inserted in to a vein on the anterior aspect of the elbow while participants were in the supine position. Venous blood was drawn via syringe into 8 mL SST II (BD, Auckland, NZ) and 10 mL K_2EDTA (BD, Auckland, NZ) containing vacutainers. SST II vacutainers were allowed to clot at room temperature for 30 min and then centrifuged at 2500 rpm for 15 min, while EDTA vacutainers were centrifuged immediately after withdrawal. After centrifugation serum and plasma was removed and pipetted in to 300 μL aliquots before being stored at -80° for subsequent analysis. Cytokines were analysed in the pre-exercise, immediately post-exercise, 60 min and 120 min post-exercise blood samples, while serum hormones were analysed in the pre-exercise, immediately, 15 min, 30 min, 60, 120 min and 840 min post-exercise blood samples (Figure 4.2).

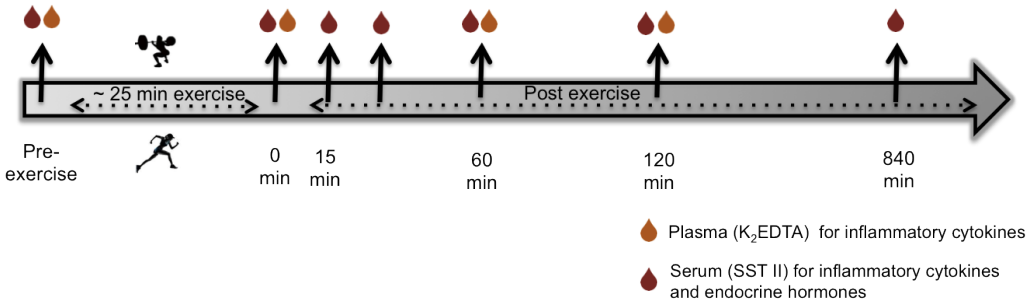


Figure 4.2: Time course of blood draws for analysis of endocrine hormones and inflammatory cytokines.

Comparison of plasma and serum for cytokine analysis

To the author's knowledge, serum versus plasma concentrations for inflammatory cytokines, have not been directly compared in exercised human blood. Due to the differences in post collection biochemical processing (serum allowed to clot at room temperature for up to 60 min, while plasma is centrifuged immediately after withdrawal) leading to subtle differences between the components of plasma (contains fibrinogen) and serum (does not contain fibrinogen), it was considered important to determine if one component of blood would maintain analyte levels better than the other, allowing for greater detection during analysis. Therefore,

cytokine measurement in human plasma and serum post-exercise was compared to determine if there were any differences in the detectable levels of inflammatory cytokines in plasma and serum samples. Plasma and serum cytokine concentrations were analysed using a high sensitivity human cytokine magnetic bead panel (MILLIPLEX MAG, HSCYTMAG-60SK, EMD Millipore Corporation, Billerica, MA, USA). Plasma and serum samples for 5 participants were analysed in concomitant plasma and serum samples according to the manufacturer's instructions except samples were analysed in singleton. Samples were measured using Luminex xMAP technology (Luminex Corporation). The multiplex assay was used for the simultaneous quantification of the following inflammatory cytokines: IL-1β, IL-8, IL-10, IL-6 and TNFα. Intra-assay coefficients of variation (CV) were under 5 %. Due to the allocation of resources to the comparison of plasma and serum cytokine concentrations, post-exercise cytokine responses were only able to be measured in 5 participants.

Serum hormone analysis

Serum samples were analysed for cortisol, IGF-1, and GH using commercially available (DRG International, Inc., USA) enzyme-linked immunosorbent assay (ELISA) kits. Serum hormones were measured in duplicate according to the manufacturer's instructions. All intra-assay CV's for these hormones were under 5 %.

Statistical analysis

For the purpose of serum versus plasma comparison of absolute values sprint and resistance data were pooled and paired t tests were run on the data to determine if significant differences existed in the detectable values of serum and plasma. To determine the effect of exercise on inflammatory cytokine and hormone responses a linear mixed model was performed for each dependent (cytokines and hormones) variable, with exercise mode and time as fixed factors, and participants as a random factor. As hormone and cytokine variables were positively skewed, a log transformation was applied to these data prior to conducting statistical analysis on means. Area under the curve (AUC) was calculated by trapezoid method for hormones and cytokine data inclusive of the 30 min of the exercise protocol plus 120 min of recovery. AUC was log transformed and compared between RSA and RT exercise by paired t test. Average exercise HR and RPE were compared between exercise trials by paired t test. For all analyses differences were considered significant at the $p < 0.05$ level. Data are presented as means $\pm$ SD.

Results

Results for serum and plasma cytokine comparison

Plasma showed significantly higher detectable levels of IL-6 (plasma: 6.79 ± 5.45 vs. serum: 3.55 ± 3.02 pg/mL, $p = 0.002$) and IL-10 (plasma: 110 ± 87.08 vs. serum: 30.17 ± 24.77 pg/mL, $p < 0.01$) when compared to serum (Figure 4.3). There were no significant differences in the detectable levels of IL-8 (plasma: 5.92 ± 8.87 vs. serum: 7.24 ± 5.05 pg/mL, $p = 0.43$) or IL-1β (plasma: 3.21 ± 5.59 vs. serum: 2.65 ± 4.63 pg/mL, $p = 0.52$) between serum and plasma samples. However, TNF-α produced significantly lower detectable levels in plasma compared with serum (plasma: 9.52 ± 4.03 vs. serum: 13.4 ± 3.51 pg/mL, $p < 0.01$). Due to the higher detection/concentrations of the main inflammatory responsive cytokine IL-6 and anti-inflammatory cytokine IL-10 in plasma compared with serum, quantified plasma samples were used for the subsequent analysis of the post-exercise and recovery cytokine response between isolated RSA and RT exercise in the current study and subsequent inflammatory cytokine analysis in proceeding experimental chapters.

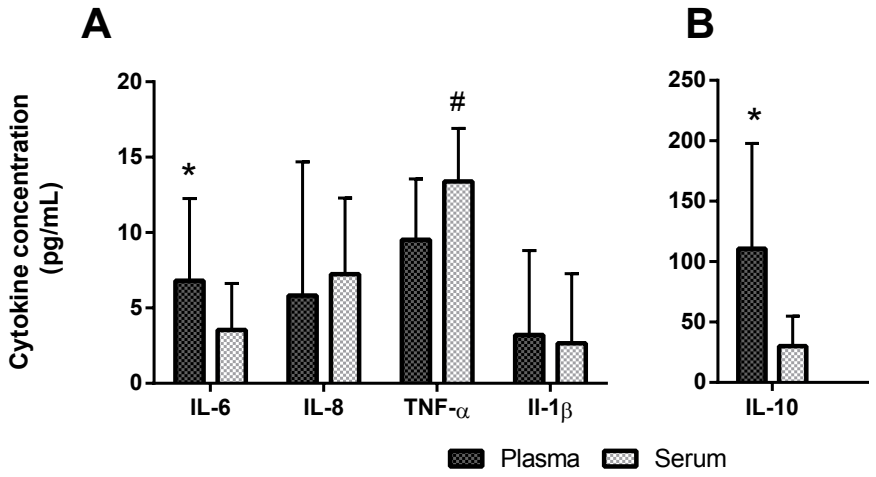


Figure 4.3: Comparison of the detectable levels of A) IL-6, IL-8, TNF-α, IL-1β, B) IL-10 in serum and plasma samples. *Significantly higher than serum, $p < 0.05$, #Significantly higher than plasma, $p < 0.05$. Data presented as means ± SD.

Heart rate and rating of perceived exertion

The effects of the RSA and RT exercise on HR and RPE are summarised in Table 4.1. Average exercise HR was significantly higher during the RSA protocol than during the RT protocol (RSA: 174 ± 6 vs. RT: 126 ± 16 $beats \cdot min^{-1}$, $p < 0.001$). However, average exercise RPE were not significantly different between the RSA and RT trials (RSA: 16 ± 2 vs. RT: 15 ± 1, $p = 0.093$).

Inflammatory cytokines

The effects of RSA and RT exercise on inflammatory cytokines are summarised in Figure 4.4. No statistically significant differences from pre-exercise were achieved for IL-8, IL-10, IL-1β or TNF-α following either mode of exercise ($p > 0.05$). However, IL-6 was found to be significantly higher than pre-exercise at 120 min in to recovery regardless of exercise mode (pre-exercise: 4.59 $\pm$ 2.92 vs. 120 min post-ex: 9.40 $\pm$ 7.32 pg/mL; main effect for time, $p = 0.033$). AUC did not differ significantly between the RT and RSA trials for IL-6 ($p = 0.98$), IL-8 ($p = 0.67$), IL-1β ($p = 0.41$), IL-10 ($p = 0.84$), and TNF-α ($p = 0.68$).

Table 4.1: Heart rate and ratings of perceived exertion during the RSA and RT protocols.

	Ex.Order	Rest	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Av.
HR	RSA	60 (7)	169 (6)	174 (6)	176 (6)	178 (5)			174 (6)$^{\#}$
$(beats \cdot min^{-1})$	RT	59 (8)	126 (19)	127 (20)	129 (22)	133 (20)	132 (21)	133 (22)	126 (16)
RPE	RSA		14 (2)	15 (2)	16 (2)	18 (2)			16 (2)
(6-20 scale)	RT		13 (1)	14 (1)	14 (1)	15 (1)	15 (1)	16 (2)	15 (1)

Data presented as mean (SD). $^{\#}$Significantly different from RT.

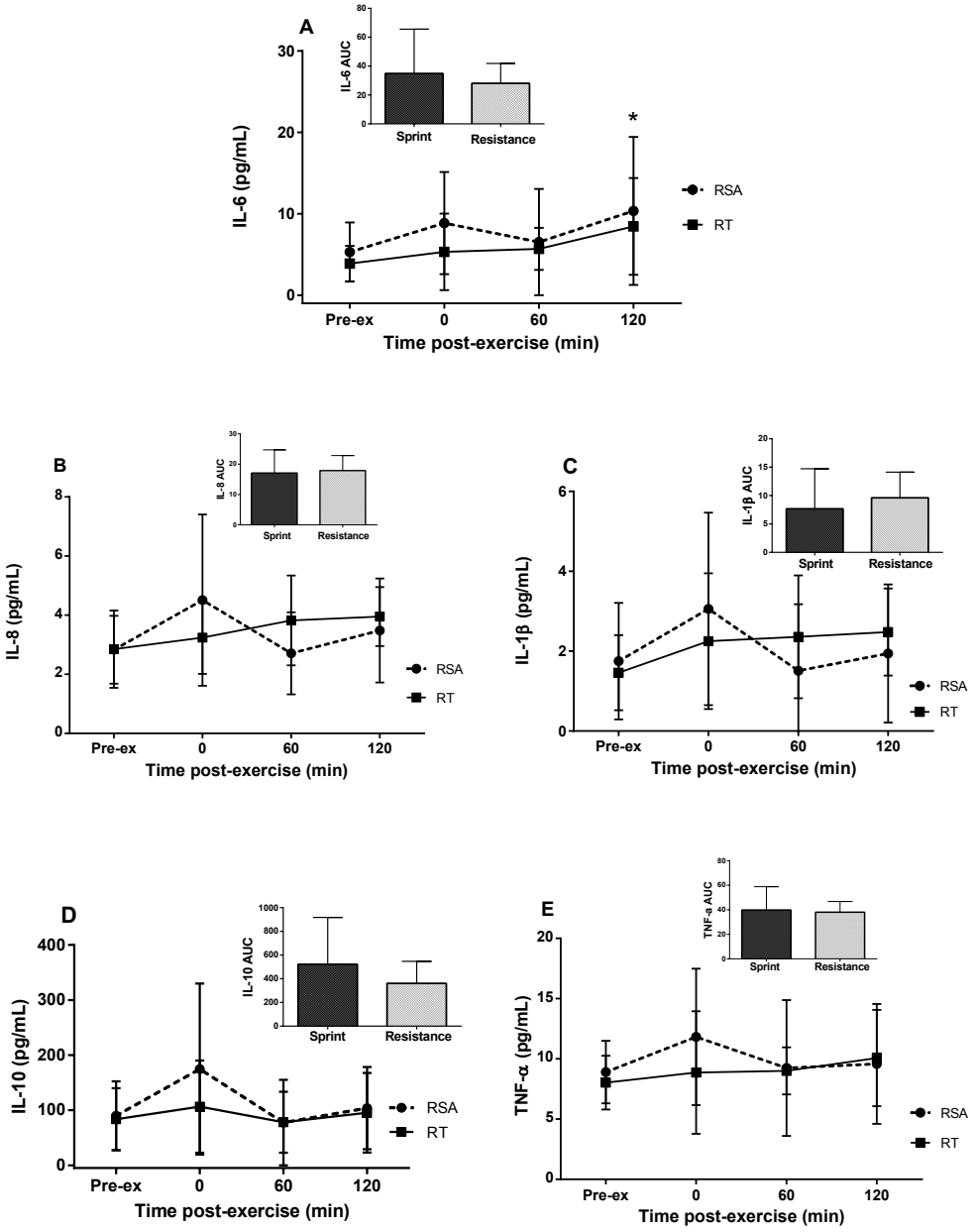


Figure 4.4: Inflammatory cytokine responses (A) IL-6, (B) IL-8, (C) IL-1β, (D) IL-10, (E) TNF-α to RSA and RT exercise, $n = 5$. *Significantly different from pre-exercise; main effect for time, $p < 0.05$. Data presented as mean ± SD.)

Endocrine hormones

The effects of the RSA and RT exercise on the endocrine hormones are summarised in Figure 4.5. Exercise of either mode was not associated with a significant increase from pre-exercise in GH in the immediate recovery period, while GH concentration did drop below pre-exercise levels at 60 min into recovery (pre-exercise: 6.64 ± 4.30 vs. 60 min post-ex: 4.17 ± 3.93 ng/mL; main effect of time, $p < 0.01$) and remained

below pre-exercise levels at 840 min in to recovery regardless of exercise trial (4.08 ± 5.40 ng/mL; main effect of time, $p = 0.002$). There was no difference in GH AUC between the RSA and RT trials. Serum cortisol concentration remained unchanged from pre-exercise within 30 min of recovery before significantly dropping below pre-exercise levels at 120 min into recovery (pre-ex: 133.41 ± 49.11 vs. 120 min post-ex: 108.10 ± 43.22 ng/mL; main effect of time, $p = 0.007$) regardless of exercise trial. At 840 min post-exercise cortisol was significantly higher than pre-exercise levels in both trials but was significantly lower at 840 min following the RSA trial compared with the RT trial (RSA: 269 ± 89.49 vs. RT: 369.85 ± 116.65 ng/mL; trial × time interaction, $p = 0.03$). There was also a strong trend for cortisol AUC to be higher in the RSA trial when compared to the RT trial (RSA: 697.17 ± 206.92 vs. RT: 515.23 ± 171.89 ng·min·mL^{-1}, $p = 0.052$). There were no significant effects of exercise on serum IGF-1 levels.

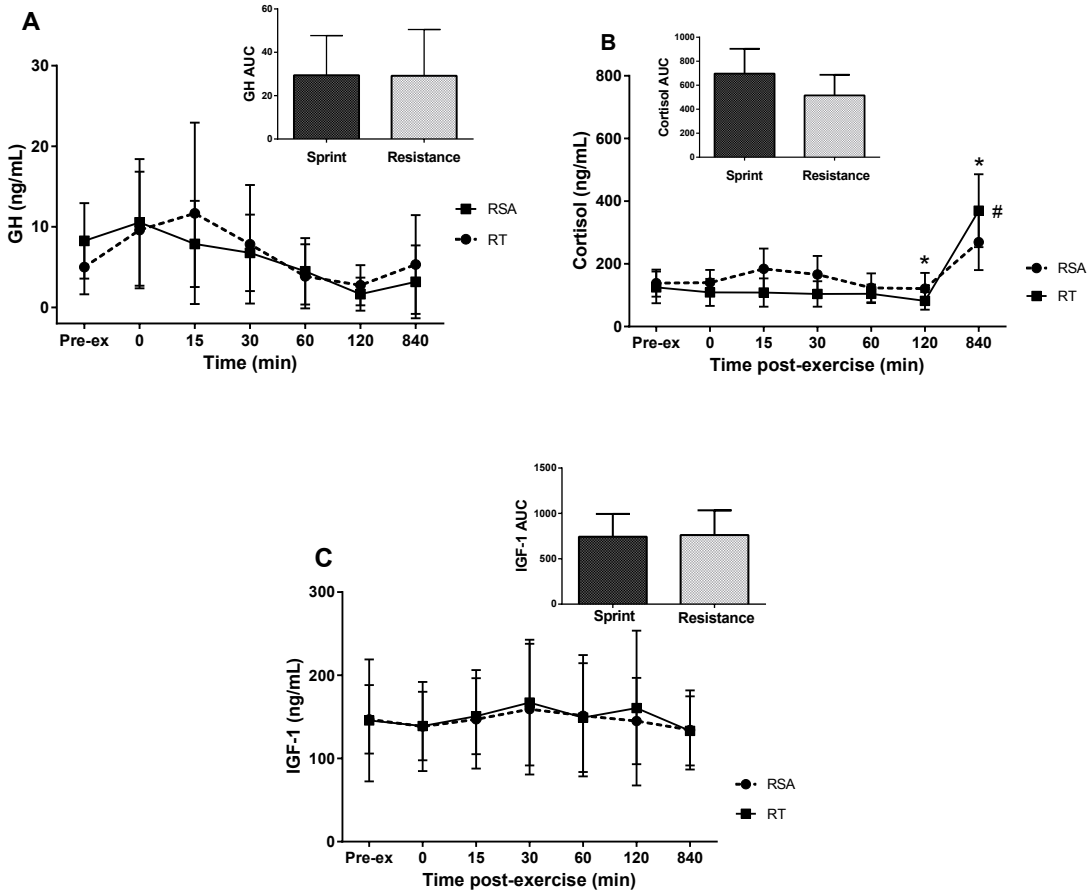


Figure 4.5: Endocrine (A) GH, (B) Cortisol, (C) IGF-1) hormone responses to RSA and RT exercise in serum, $n = 8$. *Significantly different from pre-exercise; main effect for time, $p < 0.05$. #Significantly different from RT, $p < 0.05$.

Discussion

In the present study, the effects of short duration RSA exercise and short duration RT exercise on circulating inflammatory cytokines and endocrine hormones in trained female team-sport athletes were compared. Both RSA and RT exercise was associated with a significant increase in plasma IL-6 from pre-exercise levels at 120 min into recovery. Cortisol concentration was lower than pre-exercise levels by 120 min following either exercise mode which was accompanied by a significant drop in GH below pre-exercise levels. By 840 min into recovery, natural circadian rhythm had increased cortisol concentration significantly above pre-exercise levels but cortisol concentration was significantly lower following RSA exercise compared with RT exercise at 840 min in to recovery. Exercise appeared to have little effect on circulating IGF-1, TNF-α, IL-1β, IL-10 or IL-8 levels within 120 min following exercise.

It is well known that IL-6 appears to be able to influence the hypothalamo-pituitary-ardrenal axis (HPA) [288]. A dose dependent relationship between IL-6 and cortisol has been reported in non-exercising humans [183, 289] via increases in adreno-corticotrophin hormone [289]. However, the circulating concentrations of plasma IL-6 during these studies peaked between 50 pg/mL and 255 pg/mL. During exercise, free of biological stimulation (infusion of IL-6, catecholamines or glucocorticoids) a cortisol-IL-6 relationship is less clear. In those studies that have investigated but did not observe a cortisol-IL-6 relationship, intermittent or resistance type exercise was performed and low absolute and relative changes in IL-6 concentration (< 5 pg/mL) were recorded [290,291]. Even when 2.5 h of running exercise increased IL-6 concentration to 80 pg/mL, there was no significant correlation with cortisol [160]. So while modest-high levels of IL-6 may be able to cause an increase in cortisol, levels higher than $\sim$80 pg/mL appear to be required and are likely associated with severe metabolic stress during exercise. Although long duration exercise has been shown to increase IL-6 above 100 pg/mL [133, 160], in the current study a peak IL-6 concentration of 10.4 pg/mL was achieved. Interestingly in the current study, IL-6 peaked at 120 min following exercise, which coincided with a significant drop in cortisol below pre-exercise levels supporting the idea that perhaps an IL-6/cortisol relationship is not apparent at lower concentrations of systemic IL-6.

Therefore, in the current study, the duration and intensity of exercise may not have been adequate enough to significantly increase metabolic demand and therefore IL-6 may not have reached sufficient circulating concentrations to significantly stimulate the HPA and release of cortisol. The significantly lower cortisol concentration at 840 min following exercise in the RSA trial may indicate suppression of catabolic mediators to enable an environment more conducive to muscle repair and regeneration indicating the eccentric component of the RSA (deceleration phase of

maximal sprint) exercise may have caused slightly more muscle disruption than the RT exercise, however this is only speculation.

IL-6 is thought to be an inflammation responsive cytokine that mediates the inflammatory response to exercise that may aid tissue adaptation and repair following muscle contraction [292, 293]. It has also been shown that the major source of IL-6 during exercise is thought to be the skeletal muscle, which is also hypothesised to release IL-6 in response to lowering muscle glycogen stores possibly to stimulate the release of glucose from the liver. [146, 279]. The greater IL-6 response immediately following RSA exercise (though not statistically significant) may be suggestive of greater metabolic stress compared with the RT exercise, however this is only speculation. The inflammatory cytokine response to exercise has shown to be regulated by both duration and intensity of exercise [31–34], and therefore it appears likely that the combination of these factors within the current study for either RT or RSA exercise were not sufficient to elicit significant elevations in these bio-markers. As IL-6, the inflammatory mediator cytokine, was not significantly elevated above pre-exercise concentration until 120 min in to recovery, this likely provides an explanation as to why the other anti- or pro-inflammatory cytokines were not elevated within this period.

Exercise was not associated with any significant increases in GH or IGF-1 while there was a strong trend for cortisol AUC to be higher in the RSA compared with the RT trial. The lack of response in the endocrine system within the present study was unexpected as both resistance exercise [294, 295] and sprint exercise [43, 296] have been shown to be potent stimuli for GH release. However, RT bouts that contain larger volumes of total work, (e.g., full body exercises that activate large volumes of skeletal muscle at multiple sets of 10-RM) and shorter rest periods (< 2 min) have been shown to stimulate larger post-exercise concentrations of endocrine hormones than bouts that include higher intensity lifts (< 6 reps) separated by longer rest periods (> 3 min) [131, 295]. Therefore, it may be that in the current study the volume of exercise was not large enough to evoke a significant response from the endocrine system.

Previous studies have examined the hormonal response to repeated-sprint type exercise which have been shown to be an effective stimulus for components of the GH-IGF-1 axis [43, 98, 296]. The duration and distance of these previously studied repeated-sprints were far greater than the duration of the sprints in the current study (30 s, 100-400 m) and therefore likely created considerable metabolic disturbance. It was felt that the RSA protocol employed in the current study was more reflective of best practice for team-sport athletes and even though more maximal sprints were completed in the current study, the shorter duration of sprints ($\sim$5 s) interspersed with $\sim$15 s rest between reps and 3 min rest between sets was likely well

tolerated by the glycolytic system. Further the repeated-sprints in the current study were similar to what team-sport athletes would encounter during match-play [118] and could be considered accustomed exercise. Accustomed exercise may reduce the metabolic demands and exercise may thereby fail to provide a sufficient stimulus for the endocrine system. Training status has been shown to have a large influence on the magnitude of the adrenal cortical response with trained individuals presenting a significantly lower acute response in trained compared with untrained participants [297, 298]. Therefore, in the current study, volume of exercise and training status may explain why there was only a small endocrine response to both short duration RSA and RT exercise.

Interestingly, many studies provide no explanation as to why they have chosen the component of blood they have for the analysis of systemic cytokines. Due to differences in biochemical processing of plasma and serum, the short half life of IL-6 (6 mins; personal communication Greg C. Smith) and the length of time that serum requires to clot, it was important to determine if there were differences between the detectable levels of cytokine concentration in serum and plasma. Interestingly, this study found significant differences in the detectable levels of IL-6, IL-10 (higher in plasma) and TNF-α (higher in serum) in plasma and serum following exercise. Therefore, future researchers looking to measure inflammatory cytokines during exercise should take the component of blood for analysis in to account prior to blood collection. These results suggest that caution should be taken when trying to compare post-exercise cytokine responses between measures taken from serum and plasma as absolute values may be equivocal. As a consequence this makes comparing similar studies currently difficult when different components of blood are used for analysis. Further investigative measures would be suggested in order to optimise systemic cytokine measurement/analysis following exercise, including whether the use of inhibitors that prevent the break down/deterioration of cytokines can further perfect detection.

In conclusion, 20 min of RSA or RT exercise did not cause large responses of either the inflammatory or endocrine systems despite significant increases in HR and RPE in trained female team-sport athletes. However cumulative cortisol response appeared to be greater following RSA, suggesting, alongside the higher cardiovascular responses, that RSA was possibly the more stressful of the two exercises. Further, lower cortisol levels at 840 min in to recovery in the RSA exercise may indicate suppression of a catabolic environment to enable muscle regeneration in the latter hours following exercise. Both modes of exercise induced a significant elevation in IL-6 from pre-exercise levels at 120 min in to recovery that was likely part of an inflammatory mechanism. It appears that the physiological tolerance of the athletes in this study to the prescribed exercise was high and likely a longer duration of

exercise is required to significantly stress an endocrine and inflammatory response in trained female athletes. As the cumulative systemic hormonal and inflammatory responses were not markedly different between exercise modes, they may pair well in a concurrent training scenario which would increase the duration of exercise and may provide a greater stimulus for training adaptation.

CHAPTER 5

Inflammatory and hormonal responses to same-session concurrent repeated-sprint and resistance exercise in female team-sport athletes: the exercise order effect

Abstract

Despite the wealth of knowledge regarding the physiological effects of traditional endurance and resistance training, there is little information regarding team-sport specific concurrent repeated-sprint ability (RSA) and lower-body resistance (RT) training. Further the intra-session exercise sequence may be of importance when evaluating the physiological response to an acute training session. Therefore the purpose of this study was to examine the responses of a global network of inflammatory and hormonal bio-markers to concurrent RSA and RT exercise of differing orders. The effects of the intra-session exercise order on performance of each mode of exercise was also investigated. Eight well-trained female team-sport athletes (21-28 y) completed 2 trials separated by 7 days. Each trial consisted of RSA exercise (4 $\times$ 6 sets of 20 m maximal shuttle sprints, sets separated by 3 min rest), 15 min of passive rest and resistance exercise (6 $\times$ 6 sets of back squat exercise at 80 % 1-RM, sets separated by 3 min rest) or vice versa. Blood samples were collected pre-exercise, 0 min, 15 min, 30 min, 60 min, 120 min and 840 min post-exercise. Concurrent exercise of both orders significantly increased growth hormone (GH) and cortisol (C) above pre-exercise at 0 min and 15 min following exercise ($p < 0.05$), while GH cumulative response (area under the curve (AUC)) was greater in the RT:RSA order in the initial 60 min of recovery (RT:RSA: 55.04 $\pm$ 35.99 vs. RSA:RT 27.79 $\pm$ 17.93 $p < 0.000$). Increases in a number of pro- (Interleukin (IL)-2, IL-12, TNF-α, IL-8,

L-1β, IFN-γ) and anti-inflammatory (IL-4, IL-10, IL-5, IL-7, GM-CSF) cytokines as well as the inflammatory mediator IL-6 were elevated above pre-exercise levels within the 120 min recovery period regardless of exercise order ($p < 0.05$), while IL-12, IL-7, GM-CSF, IL-5 and IL-10 were higher at 120 min post-exercise in the RT:RSA order ($p < 0.05$). However, there were no differences in cumulative (AUC) response between the exercise orders for any of the cytokines. Therefore, exercise order had only a small effect on the physiological responses to concurrent RSA and RT exercise, however a more favourable cumulative GH response in the RT:RSA may suggest greater potential for anabolic adaptation.

Introduction

Repeated-sprint ability (RSA) [299] and strength and power [122,300] are considered to be of importance for athletic performance in team-sport athletes. Combining both modes of exercise in to the same training session may be effective when training time is limited. However, little attention has been directed towards the importance of the intra-session loading order within a concurrent regime on the acute physiological response. Further a greater knowledge of the physiological stress responses to specific exercise in a trained female population is required.

The combination of two different modes of exercise within the same session or training programme is known as concurrent training. Traditionally, literature investigating concurrent training have performed continuous or interval type endurance exercise alongside various models of strength training [17, 18, 50, 301–303]. While concurrent training studies have produced inconsistent results due to differing protocols and durations of training, the most predominant finding appears to be that inferior gains are achieved in strength and power compared with strength training in isolation [17,18,301]. While endurance performance may also be dampened with concurrent training [50,302], others have found no interference effects [20,46,47]. A meta analysis examining the effect of concurrent endurance and resistance training on muscular strength gains found that strength and accompanying hypertrophy were not significantly different between concurrent and strength only groups, but that muscular power was compromised in the concurrent training group [125]. Therefore, power adaptation may be more susceptible to an interference effect during concurrent training.

For many sports, RSA has an integral place in training programmes due to the specificity to the requirements of competition, particularly in a number of team-sports. However, unlike more traditional concurrent training there appears to be little research pertaining to the effectiveness of RSA and concurrent RT training. Due to the potential similarity in the work:recovery ratio of RSA and lower body

RT exercise, these two modes of exercise may potentially work well in a concurrent set-up. However, the intra-session order of these two modes of exercise may be important if there is a difference in systemic response following exercise or inability to maximally perform one mode of exercise if it is preceded by the other. Traditional concurrent exercise studies provide evidence that the first mode of exercise may well interfere with both the performance [304] of and the adaptation to the second mode of exercise [95, 96, 98].

Strenuous exercise can cause mild injury or micro-trauma within the skeletal muscle with the potential for a local and systemic inflammatory response [305]. An increase in a battery of both pro- and anti-inflammatory cytokines in the circulation have been observed following various exercise protocols [43, 133, 145]. Yet to the author's knowledge, the cytokine response following concurrent RSA and RT training has not been documented. Further, modifications in hormones of the growth hormone↔insulin-like growth factor-1 (GH↔IGF-1) axis, and the stress hormone cortisol can occur simultaneously to the changes in inflammatory cytokines [43, 44]. The evaluation of the inflammatory and hormonal response to acute concurrent exercise may provide information about the physiological stress and/or adaptation in the immediate recovery period, and whether these responses could be affected by the order of the exercise. The duration, intensity, mode of exercise and training status of participants are all likely to combine to affect the components of the inflammatory [159] and endocrine systems.

Determining the optimal order in which to perform RSA and RT exercise, in so optimising the adaptive environment, could aid the prescription of training. Thus, the current study aimed to address the order effect of the acute cytokine and endocrine hormone responses following a combined RSA and RT training session in trained female team-sport athletes. It was also aimed to determine if the first mode of exercise would have an interference effect on the ability to maximally perform the second mode of exercise. It was hypothesised that a combined training session consisting of short duration RSA exercise and short duration lower body RT exercise would be a potent enough stimulus to elevate circulating endocrine and inflammatory mediators above pre-exercise values, further it was hypothesised that this response would differ depending on the arrangement of exercise within the session.

Methods

Overview of experimental protocol

Prior to beginning the experimental component of the study participants had the aims of the research explained and signed informed consent. A 1-repetition maxi-

mum (1-RM) test for the back squat exercise was performed 1 week prior to commencement of experimental trials in which the individual load to be lifted during the experimental trials (80 % 1-RM) was calculated. Following the 1-RM test, familiarisation of the RT and RSA protocols were completed. Participants also completed the Yo Yo intermittent recovery test (YYIRT1) in the week prior to the first experimental trial as a measure of aerobic fitness. Participants were required to complete 2 experimental trials consisting of RT and RSA exercise with trials differing only by order of exercise which was randomly assigned. The total concurrent training session lasted approximately 75 mins and total absolute load and number of sets completed were exactly the same in both orders. Experimental trials took place between 17:30 and 21:00 h and were preceded by a standardised meal and 4 h fast. Blood draws for serum and plasma were taken at pre-exercise, immediately post-exercise (0 min), 15 min, 30 min, 60 min, 120 min and 840 min during recovery (Figure 5.1). All experimental procedures were approved by the Auckland University of Technology Ethics Committee and conformed to the declaration of Helsinki.

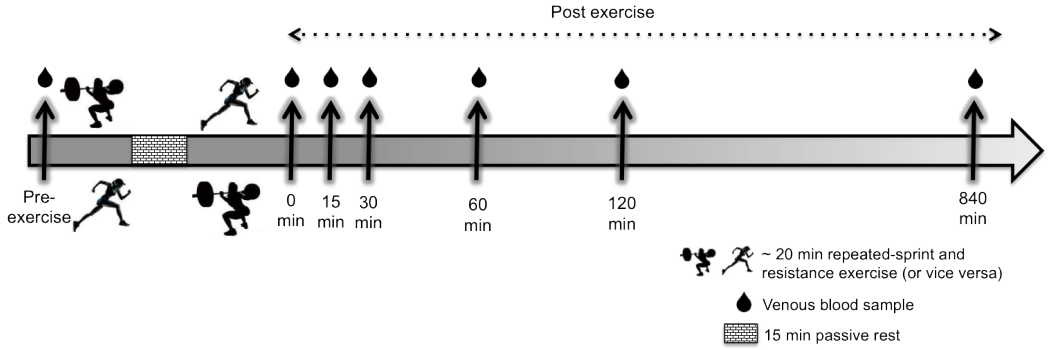


Figure 5.1: Overview of experimental protocol.

Participants

Eight female team-sport athletes (soccer, hockey, netball) [mean (SD): age 23.4 (3.2) y; body mass 68.4 (6.9) kg; $\dot{V}O_2$max 41.7 (4.8) mL·kg^{-1}·min^{-1}; 1-RM 71.5 (9.3) kg] participated in the current study. Participants were regular top level club team-sport athletes who trained with their teams at least 3 times per week. Additionally participants were also experienced with lower body resistance training but not necessarily at the same intensity (80 % 1-RM) as that employed in the current study. The study was undertaken within-season and therefore participants were considered to be at peak fitness.

Participant controls

Menstrual status and contraceptive controls

All participants were required to be taking a combined monophasic oral contraceptive (OC) pill (containing Levonorgestrel 0.15 mg and Ethinyloestradiol 0.03 mg) for the duration of the study and only completed experimental trials while taking hormonal pills. Prior to beginning OC use participants were required to have had a regular menstrual cycle.

Dietary and exercise controls

To limit confounding effects of diet and exercise on either the hormonal or inflammatory response to the experimental protocol, participants were not to perform any exercise within 24 hours of experimental trials. Participants were required to record a food diary on the day of and 1 day prior to the experimental trials and were asked to eat the same diet prior to both experimental trials. Further, participants were required to consume a standardised meal (∼51 KJ energy per kg of body mass; ∼78 % carbohydrate (CHO), ∼8 % Fat, ∼14 % Protein) 4 h prior to experimental trials. Following dinner on the night of the trial, participants were asked to refrain from eating again until after the 840 min blood sample. Participants were not permitted to consume caffeine or alcohol within 24 h of experimental trials. Participants were instructed that recovery from exercise should consist of passive rest only and that no other recovery modalities were to be used (i.e. the use of massage or anti-inflammatory products).

Preliminary testing

Familiarisation and 1-RM testing

Participants were required to undergo a 1-repetition maximum (1-RM) test for the back squat exercise as outlined by Vingren et al. 2008 [284] for the determination of the load to be lifted during the experimental RT trial (80 % 1-RM). Determination of 1-RM began after participants performed squats for 8-10 repetitions at 50 % of their estimated 1-RM followed by another set of 2-5 repetitions at 85 % of estimated 1-RM. Following on from this warm up, 4 to 5 1-repetition trials were used to determine the 1-RM. Lifts were considered successful once the top of the quads had descended to be parallel with the floor. The reliability of this test in our laboratory is high, intraclass correlation coefficient (ICC) = 0.97 and coefficient of variation (CV) = 2.5 %.

A familiarisation of the RT and RSA protocols were also conducted. Participants completed 50 % of each protocol so as to understand the procedures involved with each exercise mode and ensure maximal effort during experimental trials.

Yo Yo intermittent recovery test level 1 (YYIRT)

The level 1 version of the YYIRT was completed following the protocol previously described by Krustrup et al. [285]. Heart rate (HR) during the YYIRT test was measured with a RS800CX Run polar HR monitor (Polar, Kajaani, Finland) and maximum HR achieved during the YYIRT test was recorded. Distance covered during the YYIRT test was converted to an estimated $\dot{V}O_2$max score [286]. Female athletes who achieved an estimated $\dot{V}O_2$max score above 40 $mL \cdot kg^{-1} \cdot min^{-1}$ were included in the current research.

Experimental procedure

Each participant performed 2 exercise trials that were separated by 7 days and consisted of either RSA followed by a 15 min passive rest and then by RT (RSA:RT) or the vice versa (RT:RSA). Each exercise trial consisted of the same exact volume of exercise and differed only by exercise order. Exercise trials were completed from 17:30 to 19:00 h.

Repeated-sprint protocol

Prior to beginning the RSA exercise a standardised warm-up consisting of 6 submaximal sprint efforts followed by 6 progressive sprints performed at ∼60, ∼70 and ∼80 % of maximal effort were completed irrespective of exercise order. The RSA protocol consisted of 4 sets of 6 × maximal 20 m shuttle sprints that required participants to accelerate from a standing start and sprint 10 m before turning and sprinting maximally back to the starting point. Participants were required to sprint every 20 seconds until 6 maximal shuttle sprints had been completed. Electronic timing gates (Speed Light Sports timing system, SWIFT, NSW) were used to record velocity and time(s) of each sprint. Three min of passive rest separated each sprint set. Relative (percentage decrement over the repeated efforts) RSA fatigue score was calculated for each of the 6 × 20 m sprint sets, using the percentage decrement method [306]. The reliability for total sprint time in our laboratory is high with ICC = 0.95 and CV = 1.7 % and reliability for total sprint decrement across all sets was ICC = 0.79 and CV = 14.5 %.

Resistance protocol

Prior to beginning the RT exercise irrespective of order, a standardised warm-up consisting of 1 set of 6 repetitions with the 20 kg standard Olympic bar followed by 1 set of 6 repetitions with 50 % and 1 set of 6 repetitions with 80 % of the load that was to be used for the experimental protocol. The experimental resistance protocol consisted of 6 sets of 6 repetitions of back squat exercise at 80 % of 1-RM. Sets were separated by 3 min of passive rest. A linear position transducer (Celesco, Chatsworth, CA, USA) was attached to the Olympic bar and was interfaced with specifically designed software (Ballistic Measurement System, Fitness Technology, Australia) that allowed for direct measurement of velocity-time characteristics as outlined by McGuigan et al. [307]. Peak velocity of displacement during the concentric phase of each squat was recorded and subsequently the mean peak lifting velocity for each set was determined as an average value of the peak velocity obtained in 6 squat repetitions. The detriment in peak lifting velocity across all sets (from set 1 - set 6) was calculated using the same formula used for sprint decrement.

Heart rate and rating of perceived exertion

HR was measured with a Polar RS800 watch for the duration of both experimental trials. HR was recorded pre-exercise and immediately post each of the resistance sets and each of the sprint sets. Participants were also asked to rate how hard they found each of the resistance and sprint sets by referencing the 15 point (6-20) rating of perceived exertion (RPE) scale [287].

Blood collection and analysis

Blood collection

With the participant lying in the supine position an indwelling cannula was inserted in to the antecubital vein. Venous blood was drawn via syringe in to 2 × 8 mL SST II containing vacutainers and 1 × 10 mL K_2EDTA (BD, Auckland, NZ) containing vacutainer for the collection of both serum and plasma pre-exercise, immediately post-exercise 1, immediately post-exercise 2 (0 min), 15 min, 30 min, 60 min, 120 min and 840 min post-exercise. Serum was allowed to clot for 30 minutes before being spun at 2500 rpm for 15 min. Plasma was immediately spun at 2500 rpm for 15 min. After centrifugation serum and plasma were immediately alliquoted in to 1.5 mL eppendorf tubes and frozen at -80° C until required for analysis.

Serum and plasma analysis

Serum GH, IGF-1 and cortisol concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (DRG Instruments GmBH, Germany). Serum hormones were analysed according to the manufacturer's instructions and were analysed in duplicate. All inter-assay coefficients of variation (CV) for these hormones were under 5 %. Serum hormones were measured in serum samples drawn at pre-exercise, immediately post-exercise 2 (0 min), 15 min, 60 min and 840 min in to recovery (Figure 5.2). Plasma cytokine concentrations were analysed using a high sensitivity human cytokine kit (MILLIPLEX MAG, HSCYTMAG-60SK). Plasma cytokines were analysed according to the manufacturer's instructions except samples were analysed in singleton. A multiplex assay was used for the simultaneous quantification of the following inflammatory cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13 and TNF-α. Intra-assay CV's were under 5 %. Plasma cytokines were measured in plasma samples drawn at pre- exercise, immediately post-exercise 2 (0 min), 15 min, 60 min and 120 min in to recovery (Figure 5.2). Previous work in our laboratory examining the immunoendocrine responses to isolated RSA and RT exercise within the 120 min recovery period following exercise allowed for the identification of an appropriate time course for each of the above bio-markers which is reflected in the specific time points chosen for the analysis of each assay in question.

Total and differential leukocyte counts

Leukocyte numbers were measured in K_2EDTA whole blood and analysed with a haematology analyser (Ac.T^{TM} 5 diff analyser, Beckman Coulter, NZ). Leukocytes included all counts of white blood cells including neutrophils, lymphocytes, and monocytes. All counts were adjusted for changes in plasma volume prior to analysis [308]. Counts were measured at pre-exercise, post-exercise 1, post-exercise 2 (0 min), 60 min and 840 min post-exercise (Figure 5.2).

Statistical analysis

Serum hormone and plasma cytokines were analysed with a linear mixed model with exercise order and time as fixed factors, and participants as a random factor. The trapezoid method was used to calculate the area under the curve (AUC) inclusive of the 75 min exercise protocol and 120 min of recovery for cytokines and inclusive of the 75 min exercise protocol and 60 min of recovery for endocrine hormones. Differences in AUC between exercise orders were analysed by paired t test. Performance measures and leukocyte differential counts were analysed with a two-factor (exercise

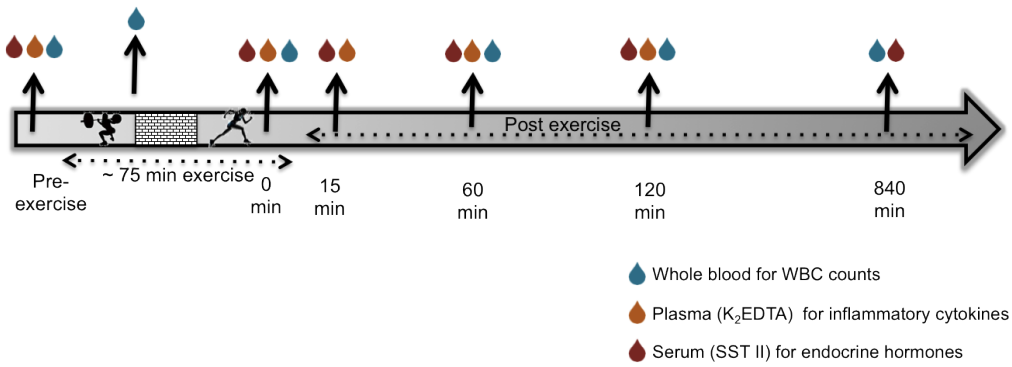


Figure 5.2: Time course of blood draws for analysis of endocrine hormones, inflammatory cytokines and total and differential leukocyte counts.

order × time) ANOVA with repeated measures. Where significant main and interaction effects were identified, Holm-Bonferroni post hoc tests were used to determine the strength of differences. Differences in average exercise HR and RPE between exercise orders were analysed by paired t test. For positively skewed distributed variables, log-transformation was performed prior to statistical analysis. Data are presented as means ± SD. For all analyses differences were considered significant at a level of $p < 0.05$.

Results

Heart rate and rating of perceived exertion

Average exercise HR during the RT protocol was significantly higher in the RSA:RT order (RSA:RT 153 ± 16 vs. RT:RSA 141 ± 16 $beats{\cdot}min^{-1}$, $p = 0.003$). Differently, average exercise HR during the RSA protocol was higher in the RT:RSA order (RT:RSA 184 ± 8 vs. RSA:RT 181 ± 10 $beats \cdot min^{-1}$, $p = 0.024$). However, average RPE was not different between orders for both RSA (RT:RSA 14.7 ± 0.6 vs. RSA:RT 15.3 ± 1.1, $p = 0.132$) and RT (RT:RSA 16.6 ± 0.9 vs. RSA:RT 16.3 ± 1, $p = 0.308$) (Table 5.1).

Table 5.1: HR and RPE responses to RSA or RT exercise during concurrent RSA or RT exercise of different intra-session order.

	Exercise Order	**Ex. mode**	**Pre-exercise**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Set 6**	Average
RPE	RSA:RT	RT		13 (1)	14 (1)	14 (1)	15 (1)	16 (1)	16 (1)	15.3 (1.1)
	RT:RSA	RT		14 (1)	14 (1)	15 (1)	16 (1)	16 (1)	17 (1)	14.7 (0.6)
	RSA:RT	RSA		14 (1)	16 (1)	17 (1)	18 (1)			16.6 (0.9)
	RT:RSA	RSA		15 (1)	16 (1)	17 (1)	18 (1)			16.8 (1.8)
HR ($beats \cdot min^{-1}$)	RSA:RT	RT	65 (13)	153 (15)	153 (16)	155 (16)	153 (15)	153 (17)	150 (19)	153 (16)$^{\#}$
	RT:RSA	RT	65 (12)	136 (14)	141 (16)	141 (16)	144 (17)	142 (17)	145 (18)	141 (16)
	RSA:RT	RSA	65 (13)	179 (11)	182 (10)	183 (8)	183 (10)			181 (10)$^{\#}$
	RT:RSA	RSA	65 (12)	180 (9)	183 (9)	186 (8)	186 (9)			184 (8)

Data presented as mean (SD). $^{\#}$Significantly different from RT:RSA, $p < 0.05$.

Table 5.2: Total sprint time and fatigue index for each repeated-sprint set.

	Exercise Order	**Set 1**	**Set 2**	**Set 3**	**Set 4**
Total Sprint Time (s)	RSA:RT	33.7 (2.6)	33.7 (2.3)	33.6 (2.1)	33.9 (2.6)
	RT:RSA	33.6 (2.6)	33.9 (2.4)	34.0 (2.5)	34.1 (2.4)
Fatigue Index (%)	RSA:RT	2.2 (2.5)	3.5 (2.1)	3.1 (1.6)	3.5 (3.4)
	RT:RSA	3.1 (1.9)	3.1 (1.2)	3.5 (1.5)	3.5 (1.6)

Data presented as mean (SD).

Table 5.3: Mean peak lifting velocity for each set of back squat exercise and velocity fatigue index during concurrent RSA and RT exercise of different intra-session order.

	Exercise Order	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Set 6**	**% peak velocity dec. set 1-6**
Average lifting velocity (ms^{-1})	RSA:RT	0.63 (0.17)	0.63 (0.14)	0.63 (0.17)	0.64 (0.17)	0.64 (0.17)	0.65 (0.19)	6.39 (12.45)
	RT:RSA	0.65 (0.17)	0.67 (0.18)	0.65 (0.17)	0.61 (0.18)	0.65 (0.20)	0.65 (0.20)	11.93 (11.15)

Data presented as mean (SD).

Sprint performance

There were no effects for exercise order or set number on sprint time per bout (sum of all 6 sprints (s)) ($p > 0.05$. Table 5.2). There were also no differences for exercise order or set number on % sprint decrement for each bout ($p > 0.05$) (Table 5.2).

Resistance performance

There were no effects of exercise order on decrement of mean squat peak velocity from set-1 to set-6 (RT:RSA 6.39 ± 12.45 vs. RSA:RT 11.93 ±11.15 %, ($p < 0.05$)) (Table 5.3).

Inflammatory cytokines

Immediately post-exercise (0 min), a significant elevation occurred in the plasma concentration of the immuno-modulator IL-6, 2 pro-inflammatory cytokines: TNF-α, IL-8, and 4 anti-inflammatory cytokines: IL-4, IL-10, IL-5, GM-CSF, (Table 5.4 and 5.5) when compared to pre-exercise concentration (main effect of time, all $p < 0.05$). These cytokines remained elevated above pre-exercise levels at all time points post-exercise ($p < 0.05$). IL-7 was significantly elevated from pre-exercise levels at 15 min post-exercise ($p < 0.05$) regardless of trial (main effect of time). The pro-inflammatory cytokines IL-1β, IL-2 and IFN-γ only became significantly elevated from pre-exercise levels from 60 min post-exercise and remained elevated at 120 min post-exercise regardless of exercise order (main effect of time, $p < 0.05$). The following cytokines were significantly higher in RT:RSA exercise order compared with the RSA:RT order at 120 min post-exercise: IL-12, IL-7, GM-CSF, IL-5 and IL-10 (time × trial interaction, $p < 0.05$). Cumulative cytokine response (AUC) for inflammatory cytokines did not differ between concurrent exercise sessions irrespective of intra-session exercise order. IL-13 was not consistently detected across all samples and was therefore excluded from analysis.

Table 5.4: Anti-inflammatory cytokine response in female team-sport athletes following concurrent RSA and RT exercise of different intra-session order.

Anti-inflammatory cytokines (pg/mL)	**Exercise Order**	pre-exercise	0 min post-ex	15 min post-ex	60 min post-ex	120 min post-ex
IL-6	RSA:RT	7.44 (5.55)	10.02 (4.38)*	9.74 (4.81)*	14.45 (5.91)*	18.45 (7.84)*
	RT:RSA	7.19 (5.29)	11.42 (3.24)*	12.11 (5.33)*	14.77 (4.24)*	18.11 (4.55)*
IL-4	RSA:RT	128.79 (89.38)	159.06 (103.11)*	137.16 (84.49)	155.84 (80.70)*	159.01 (67.64)*
	RT:RSA	129.12 (96.48)	206.57 (102.97)*	178.19 (125.00)	196.40 (112.00)*	215.35 (104.66)*
IL-10	RSA:RT	77.01 (49.88)	107.49 (55.28)*	93.56 (46.66)*	106.00 (53.89)*	115.15 (52.79)*
	RT:RSA	77.53 (57.64)	124.45 (60.80)*	121.58 (77.25)*	126.87 (70.09)*	137.34 (59.73)*#
IL-5	RSA:RT	3.68 (2.67)	4.51 (2.97)*	3.93 (2.43)*	4.25 (2.64)*	4.41 (1.65)*
	RT:RSA	3.33 (2.40)	5.38 (2.98)*	5.11 (4.07)*	5.59 (4.15)*	6.08 (4.30)*#
IL-7	RSA:RT	13.18 (8.60)	16.46 (10.04)	13.83 (8.76)*	16.00 (9.50)*	15.68 (7.08)*
	RT:RSA	12.89 (8.85)	16.33 (7.87)	18.16 (10.43)*	18.12 (8.15)*	20.81 (7.76)*#
GM-CSF	RSA:RT	6.15 (4.75)	9.20 (7.34)*	8.13 (5.82)*	9.00 (7.52)*	9.08 (5.06)*
	RT:RSA	6.97 (7.66)	10.51 (5.67)*	10.17 (6.40)*	10.71 (5.84)*	11.58 (4.86)*#

Data presented as mean (SD). *Significantly different from pre-exercise; main effect for time, $p < 0.05$. #Significantly different to RSA:RT; time × trial interaction, $p < 0.05$.

Table 5.5: Pro-inflammatory cytokine response in female team-sport athletes following concurrent RSA and RT exercise of different intra-session order.

Pro-inflammatory cytokines (pg/mL)	**Exercise Order**	pre-exercise	0 min post-ex	15 min post-ex	60 min post-ex	120 min post-ex
IL-2	RSA:RT	15.20 (17.70)	18.75 (20.58)	16.91 (18.05)	18.00 (21.38)*	16.77 (12.96)*
	RT:RSA	15.34 (20.86)	18.65 (16.22)	17.80 (16.00)	18.46 (12.41)*	20.10 (12.90)*
IL-12	RSA:RT	21.23 (13.54)	23.45 (9.36)	21.67 (10.87)*	23.73 (11.32)*	25.44 (11.21)*
	RT:RSA	20.00 (11.70)	26.54 (9.95)	24.90 (12.92)*	27.68 (12.89)*	29.93 (12.33)*#
TNF-α	RSA:RT	11.39 (4.31)	13.54 (4.66)*	13.21 (4.45)*	13.53 (4.79)*	14.80 (5.24)*
	RT:RSA	10.53 (4.20)	14.97 (3.79)*	13.76 (4.48)*	15.75 (4.79)*	16.06 (4.85)*
IL-1β	RSA:RT	5.80 (7.66)	5.66 (5.92)	5.98 (8.06)	4.63 (4.01)*	6.24 (7.48)*
	RT:RSA	5.45 (6.90)	6.93 (6.57)	6.80 (7.38)	7.29 (8.77)*	6.53 (4.69)*
IFN-y	RSA:RT	85.47 (61.81)	76.58 (41.13)	82.33 (62.29)	79.44 (36.34)*	84.72 (49.63)*
	RT:RSA	83.37 (68.43)	88.45 (55.48)	88.23 (58.01)	98.09 (77.59)*	88.40 (43.33)*
IL-8	RSA:RT	9.88 (5.34)	10.82 (5.09)*	10.26 (5.57)*	9.85 (4.74)*	9.90 (4.44)*
	RT:RSA	8.85 (5.80)	11.25 (6.27)*	11.26 (6.52)*	12.19 (7.15)*	11.34 (5.70)*

Data presented as mean (SD). *Significantly different from pre-exercise; main effect for time, $p < 0.05$. #Significantly different to RSA:RT; time $\times$ trial interaction, $p < 0.05$.

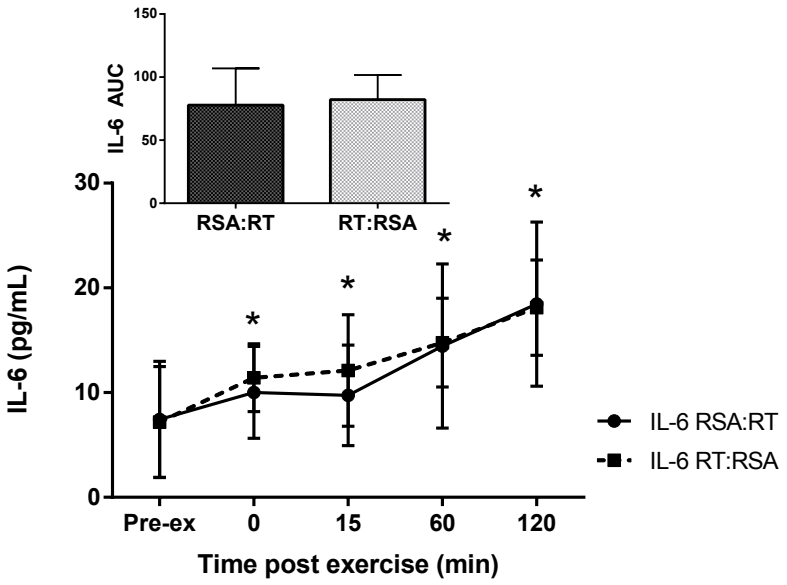


Figure 5.3: IL-6 response to concurrent RSA and RT exercise of different intra-session order. Data are presented as mean ± SD. *Significantly higher than pre-exercise values; main effect for time, $p < 0.05$.

Total and differential leukocyte count response

The white blood cell (WBC) data is summarised in Table 5.6. Total circulating leukocyte, lymphocyte and neutrophil counts were significantly elevated above pre-exercise values immediately following the first bout of exercise (exercise mode 1), (main effect for time, all $p < 0.05$). Circulating leukocyte count remained elevated at 60 min in to recovery ($p = 0.01$) before normalising to pre-exercise levels by 840 min in to recovery. Circulating leukocyte counts were significantly higher in the RSA:RT order immediately following exercise mode 1 compared with the RT:RSA order (time × trial interaction, $p = 0.01$). However, circulating lymphocyte counts remained significantly elevated after exercise mode 2 (time × trial interaction, $p = 0.009$) but were normalised to pre-exercise levels by 60 min into recovery. Circulating neutrophil counts however, remained significantly elevated at all time points failing to normalise to pre-exercise levels by 840 min in to recovery (main effects for time, $p < 0.05$). Circulating monocyte cell counts were elevated by concurrent exercise of either order 0 min post-exercise, remaining elevated at 60 min pot-exercise and were returned to pre-exercise levels by 840 min in to recovery (main effects for time, $p < 0.05$).

Table 5.6: Circulating leukocyte, lymphocyte, neutrophil and monocyte counts in female team-sport athletes following concurrent RSA and RT exercise of different intra-session order.

White Blood Cells (10^9 **cells/L)**	**Exercise Order**	**Pre-exercise**	**Immed. post-ex. 1**	**0 min post-ex. 2**	**60 min post-ex**	**840 min post-ex**
Leukocytes	RSA:RT	7.5 (1.8)	10.7 (2.4)$^{\star\#}$	9.6 (3.2)$^{\star\#}$	10.4 (3.4)*	7.5 (2.5)
	RT:RSA	7.9 (1.1)	9.1 (1.8)*	11.6 (1.7)*	10.4 (2.8)*	7.6 (2.0)
Lymphocytes	RSA:RT	3.0 (0.7)	4.6 (0.9)$^{\star\#}$	3.1 (0.9)$^{\star\#}$	3.2 (1.1)	2.6 (0.9)
	RT:RSA	2.8 (0.7)	3.2 (0.9)*	4.4 (1.0)*	2.8 (1.2)	2.3 (0.7)
Neutrophils	RSA:RT	3.3 (0.7)	4.5 (1.3)*	4.5 (2.0)*	4.7 (2.2)*	4.5 (1.7)*
	RT:RSA	4.1 (1.2)	4.3 (1.2)*	4.2 (1.3)*	5.2 (1.5)*	5.3 (2.0)*
Moncytes	RSA:RT	0.6 (0.2)	1.0 (0.2)*	0.8 (0.2)*	0.8 (0.2)*	0.6 (0.1)
	RT:RSA	0.7 (0.1)	0.8 (0.2)*	0.9 (0.4)*	0.7 (0.1)*	0.6 (0.1)

Data presented as mean (SD). *Significantly different from pre-exercise; main effect for time, $p < 0.05$. $^{\#}$Significantly different from RT:RSA; time $\times$ trial interaction, $p < 0.05$.

Endocrine hormones

Serum GH was elevated above pre-exercise levels immediately following exercise (0 min) (pre-exercise: 5.37 ± 4.38 vs. 0 min: 11.56 ± 8.89 ng/mL, main effect for time, $p = 0.001$)) and showed a trend to be elevated above pre-exercise levels at 15 min post-exercise (7.17 ± 4.27 ng/mL, $p = 0.063$), subsiding to pre-exercise levels by 60 min post-exercise irrespective of exercise order. At 840 min post-exercise, serum GH was significantly lower than pre-exercise levels (2.30 ± 3.45 ng/mL, $p = 0.003$). Serum GH AUC was higher in the RT:RSA order compared to the RSA:RT order (RT:RSA: 55.04 ± vs. RSA:RT: 35.99 vs 27.79 ± 17.93 ng·min·mL^{-1}, $p < 0.000$) (Figure 5.4). Serum cortisol was increased above pre-exercise levels immediately post-exercise (pre-exercise: 128.54 ± 34.20 vs. 0 min: 165.05 ± 63.59 ng/mL, main effect for time, $p = 0.021$), remaining elevated at 15 min post-exercise (169.32 ± 55.32 ng/mL, $p = 0.008$) before returning to pre-exercise levels by 60 min post-exercise irrespective of trial. At 840 min post-exercise cortisol was significantly higher than pre-exercise levels following both orders of concurrent exercise (327.62 ± 65.65 ng/mL, $p = 0.000$) (Figure 5.5). Exercise of either order was not associated with any significant alterations in concentration from pre-exercise values for serum IGF-1 (Figure 5.6).

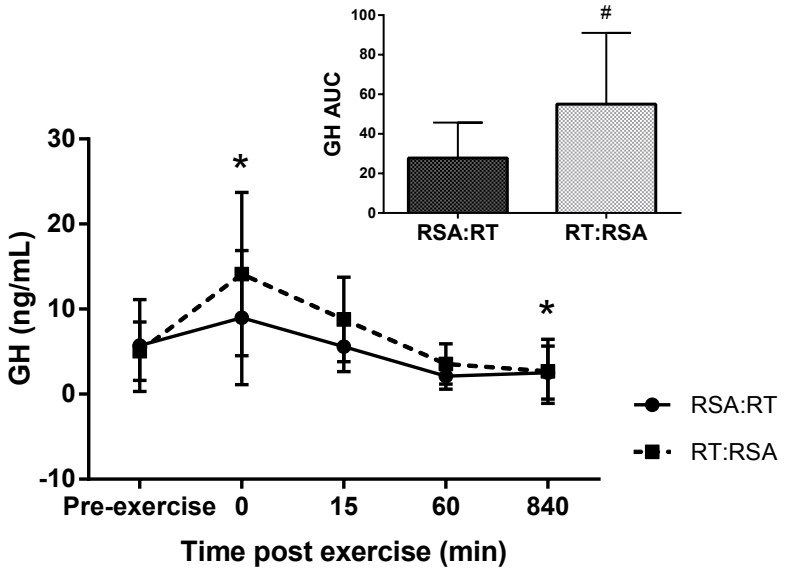


Figure 5.4: Serum GH response to concurrent RSA and RT exercise of different intra-session order. Data present as mean ± SD. *Significantly higher than pre-exercise values; main effect for time, $p < 0.05$. #Significantly higher than RSA:RT, $p < 0.05$.

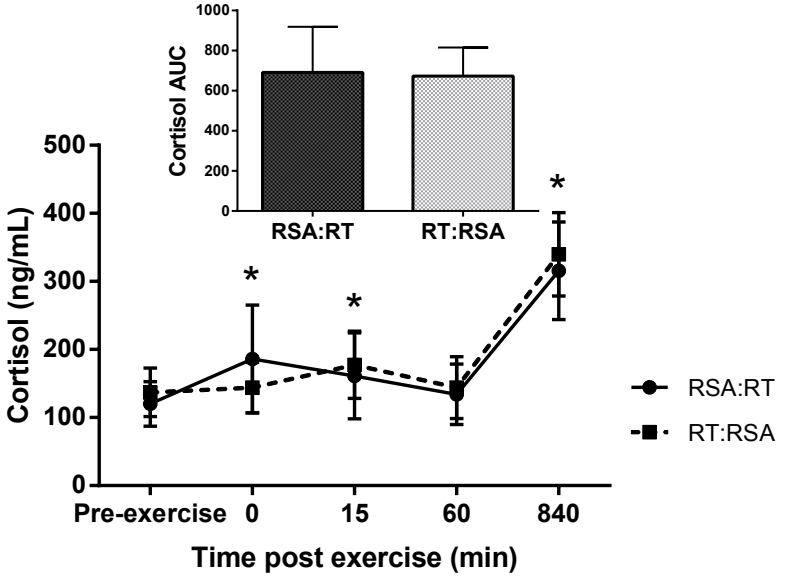


Figure 5.5: Serum cortisol response to concurrent RSA and RT exercise of different intra-session order. Data are presented as mean ± SD. *Significantly higher than pre-exercise values; main effect for time, $p < 0.05$.

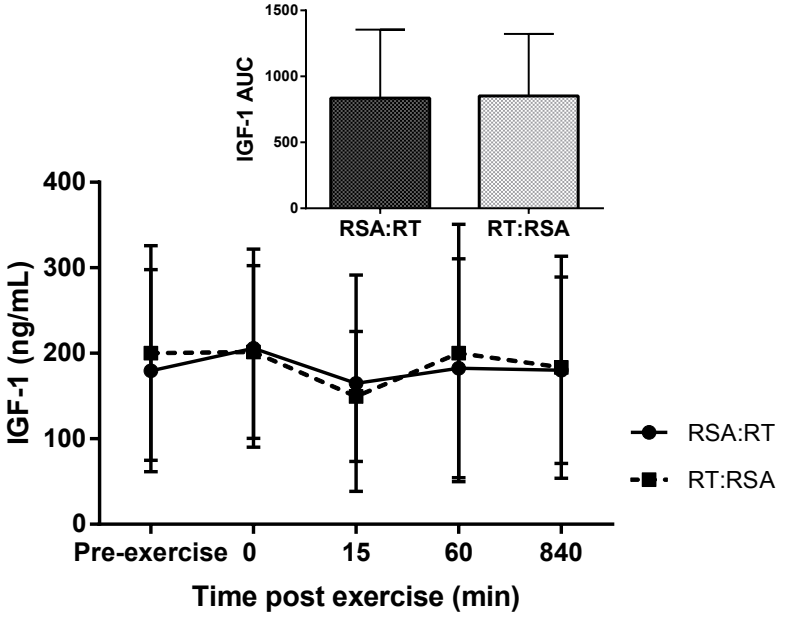


Figure 5.6: Serum IGF-1 response to concurrent RSA and RT exercise of different intra-session order. Data presented as mean ± SD.

Discussion

This study used a concurrent training session designed to stress movement patterns, muscle groups and the cardiovascular system specific to many team-sport athletes. The study design alternated the intra-session ordering of the RT and RSA exercise to assess the effects of exercise order on post-exercise bio-markers and exercise performance. The major findings of this investigation were that concurrent RT and RSA of either intra-session sequencing caused an elevation in components of both the inflammatory and endocrine systems within the immediate recovery period. Marked elevations in white blood cell count occurred immediately following the first and second exercise modes which was accompanied by an inflammatory cytokine response that was most pronounced at 120 min in to recovery. Small modifications occurred in the GH↔IGF-1 axis and circulating cortisol immediately post-exercise but these were not sustained past 15 min in to recovery. Cumulative GH response was greater in the RT:RSA order and performance of either exercise mode was unaffected by the preceding exercise.

Performance

The order of RT and RSA exercise did not appear to affect the measured performance variables (velocity for RT and sprint decrement and time for RSA exercise) nor perception of exertion (RPE). This suggests that 15 minutes of passive rest was adequate for the cardiovascular and neuromuscular systems to sufficiently recover from either mode of exercise prior to performing the subsequent exercise mode, such that no performance decrement was observed. These results are in contrast to those found by Leveritt et al. [304] who found that an acute bout of high-intensity endurance exercise caused significant reductions in isokinetic torque during full leg extension and number of isoinertial squat lifts performed in a subsequent bout of resistance activity. Similarly Reed et al. [309] found that inclusion of bicycle exercise resulted in an impaired back squat performance in terms of lifts performed in a subsequent resistance bout. However, both of these studies required participants to complete squats until volitional fatigue, whereas in the current study a set number of squats were required to be performed, and performance was measured by decrements in peak squat velocity and therefore may account for the differences seen in the subsequent resistance performance in these studies. However, the results of the current study provide no evidence to suggest that extensive fatigue from the first mode of exercise existed, nor provided detrimental effects on the subsequent mode of exercise. Therefore, it appears that lower body RT and RSA exercise performed with a similar work:rest ratio are compatible within an acute concurrent exercise regime.

Leukocyte count response

Concurrent exercise, regardless of order caused considerable leukocytosis that was elicited by significant elevations in both circulating lymphocytes and neutrophils. Peak leukocytosis occurred immediately post RSA exercise in both concurrent trials, suggesting that RSA exercise provided a greater immediate stress response for the immune system than RT exercise. This may reflect a greater influence of the stress hormone adrenaline on leukocyte mobilisation (specifically lymphocytes) immediately following high-intensity dynamic sprint exercise compared with the more locally confined muscle contractions of the resistance exercise in this study. While not measured in the current study, high-intensity aerobic/sprint type exercise is known to stimulate the release of adrenaline [310] which is associated with a marked increase in circulating leukocytes [311]. Circulating leukocyte, neutrophil, lymphocyte and monocyte post-exercise responses were also similar in pattern to those elicited by a 90 min soccer match in elite female players [145]. The magnitude of the responses, however, were higher in the present study, most likely due to the overload nature of the training stimulus, resulting from a higher exercise intensity within a shorter time-frame, necessary in order to elicit training adaptation.

Inflammatory Cytokine Response

A limitation of studies investigating the systemic cytokine response to exercise has been the analysis of only a small number of cytokines, most frequently Il-6, IL-10, IL-1β and TNF-α [135, 180]. While this still provides important information, due to the complexity of the cytokine cascade and the antagonising actions of the pro and anti inflammatory sides of the immune/inflammatory response a more global investigation was undertaken in the current study. This study observed a post-exerise inflammatory response to concurrent RT and RSA exercise characterised by a significant increase in both pro- (IL-2, IL-12, TNF-β, IL-1β, IFN-y, IL-8), and anti-inflammatory (IL-6, IL-4, IL-10, IL-5, IL-7, GM-CSF) cytokines in both exercise orders. Interestingly, the anti-inflammatory cytokines appeared to be elevated immediately by the exercise stimulus, where as the pro-inflammatory cytokines exhibited a more delayed response, elevating above pre-exercise levels at 15 and 60 min post-exercise. The overall pattern and magnitude of responses were similar between the concurrent exercise orders. While IL-12 and anti-inflammatory cytokines IL-7, GM-CSF, IL-5 and IL-10 were modestly but significantly higher at 120 min in to recovery from exercise in the RT:RSA order, there were no differences in the cumulative cytokine responses (AUC) between exercise orders. There were also no differences in cumulative WBC mobilisation, cortisol response or stress of the exer-

cise protocol (HR and RPE) between the two trials and as such makes it difficult to suggest why there were small differences in these cytokines at 120 min post-exercise.

IL-6 has been termed a central mediator of the inflammatory cascade to exercise as it is shown to precede all other cytokines, particularly the anti-inflammatory cytokine IL-10 [159, 183], (providing some evidence for an anti-inflammatory action of IL-6) which has been suggested to play a role in the up-regulation of other anti-inflammatory cytokines, namely IL-4 and IL-13 [312]. IL-6 therefore likely accounts at least partly for the increase in concentration of the global inflammatory cytokine response seen immediately following concurrent exercise in the current study. Further, IL-6 and IL-8, both of which were elevated by the concurrent exercise in this study are known to be neutrophil attractants [192] and therefore may likely explain the significant elevations in neutrophils seen within the 60 min post-exercise recovery period in the current study. It is possible that the exercise protocol in the current study caused myofibrillar disruption or micro trauma due to the downward phase of the back squats, and the deceleration phase of the shuttle sprints (eccentric contraction) that required the infiltration of neutrophils and monocytes into the skeletal muscle to mediate reparative processes [313]. Monocytes and neutrophils were still elevated in the blood stream at 60 min post-exercise, lending support to this possible explanation.

On the whole, it appears that the inflammatory cytokine response to concurrent RT and RSA exercise involved a global network of cytokines, where increases in pro-inflammatory cytokines were tightly restricted by the immediate elevations in anti-inflammatory cytokines. These observations are similar to those previously reported following both short [314] and long endurance exercise (marathon) [133] and high-intensity intermittent soccer match-play exercise in males and females [145, 272]. As this study did not measure beyond 120 min post-exercise it is difficult to ascertain how long the inflammatory cascade remained elevated and if the pro-↔anti-inflammatory balance shifted at any point following the 120 min recovery period. However, a number of cytokines measured in the current study were elevated immediately following an elite female soccer match, with all measured cytokine levels normalised to baseline by 21 h in to recovery [145], suggesting that the inflammatory response to exercise is normalised relatively promptly, or that local inflammation takes over from the systemic response within the hours following exercise. Further investigations may advance knowledge in this area by comparing systemic and skeletal muscle cytokine concentrations in the post-exercise phase within the same individuals. Comparisons of skeletal muscle and systemic cytokine concentrations and response patterns will help to determine whether the local and systemic responses are closely related, and subsequently whether the systemic response can be used indirectly to assess local inflammation.

IL-6 has also been shown to be released due to metabolic stress during exercise [33, 292] and as such may have dual mechanisms during exercise which may occur simultaneously. IL-6 is now widely accepted to be produced locally within the contracting muscle and then secreted in to the systemic circulation [136] in response to depletion of intramuscular glycogen stores [279] thus acting as an energy sensor [315]. Once in the systemic circulation it is hypothesised that IL-6 acts in an endocrine like manner, influencing hepatic glucose output in order to increase circulating glucose levels [35, 316]. Skeletal muscle glycogen is the major fuel source during exercise above 85 % $\dot{V}O_2$max in a fasted state [317]. While intramuscular glycogen stores were not measured in the current study, it can be assumed that the concurrent exercise employed, in particular the RSA exercise may have placed some demand on muscle glycogen stores resulting in mild depletion. Similar exercise to that in the current study has shown significant decreases in skeletal muscle glycogen content: 30 s treadmill [318] or cycle sprints [130] (up to 32 %), a single 6 s maximal cycle sprint (14 %) [319], 6 sets of leg extension at 35 and 75 % 1-RM (39 %) [320]. However, due to only mild endocrine stimulation/activation and minimal fatigue in performance during the current study, it would suggest that glycogen depletion did not reach critical levels. Nevertheless, it is possible that the significant elevation in IL-6 above pre-exercise levels immediately after cessation of exercise was stimulated by mild metabolic stress.

While it is likely that the increase in IL-6 in the minutes and hours following exercise is inflammatory related, it is not easy to determine whether the immediate post-exercise increase in plasma IL-6 is metabolically stimulated or the initiation of an inflammatory response or possibly that both mechanisms are simultaneously at play, making the interpretation of the immediately post-exercise systemic IL-6 response challenging.

Endocrine Response

Concurrent exercise of either order resulted in a small but significant serum GH response immediately post-exercise (0 min) which remained elevated at 15 min into recovery, returning to pre-exercise concentrations by 60 min into recovery. A significantly larger cumulative GH response within the 60 mins of recovery was observed in the RT:RSA trial which may reflect a greater anabolic hormonal response to the RT exercise which may have been suppressed when preceded by repeated-sprint exercise. There was a small increase in circulating cortisol above pre-exercise levels immediately post (0 min) and 15 min post-exercise and in a similar pattern to GH was returned to pre-exercise levels by 1 h post-exercise. Although not significantly different, peak cortisol concentration occurred at different times between exercise orders (RSA:RT = immed post-ex (0 min) vs. RT:RSA = 15 min post-exercise) ap-

pearing to be exaggerated by the RSA exercise and associated cardiovascular stress. Diurnal rhythm had significantly increased cortisol above pre-exercise levels the morning following the exercise trials, but concentrations were not different between trials. Interestingly, when these two modes of exercise were previously performed in isolation (Chapter 4), RSA was associated with a lowered cortisol response at 840 min in to recovery when compared to resistance exercise. However, upon combining both modes of exercise into a single concurrent training session, this response was abolished. In a previous study with a larger volume of resistance exercise (also in female participants), cortisol remained unchanged following 5 sets of 10 reps of 2 lower body and 1 upper body exercise (bench press, sit-up exercise and bilateral leg press) [321], supporting the results of the current study, that the RSA exercise may have had the greatest influence on cortisol levels. Despite the increases in GH and cortisol, the exercise stimulus did not appear potent enough to increase circulating levels of IGF-1 above pre-exercise levels irrespective of concurrent exercise order.

The previous study (Chapter 4) demonstrated that 4 × 6 bouts of repeated-sprint exercise or 6 × 6 sets of back squat exercise at 80 % 1-RM appeared insufficient to cause a large inflammatory or endocrine response in a similarly trained group of female team-sport players. However, by completing both modes of exercise within the same training session with only a 15 minute passive break, a marked inflammatory cascade within 120 min in to recovery, and elevated GH and cortisol concentrations within the initial 15 mins of recovery were observed. Thus, it appears that exercise duration in combination with exercise intensity may be important when it comes to stimulating the endocrine and inflammatory systems. This may be important to consider in a chronic training scenario, where combining the two modes of exercise may provide a greater positive environment for exercise induced adaptation. However, the role of acute elevations in GH or IGF-1 in anabolic adaptation to exercise is currently debated [107,108] and caution should be taken when extrapolating acute elevations in these hormones to a chronic training and adaptation scenario.

Conclusion

This study examined the inflammatory, immune, hormonal and performance responses to concurrent RSA and RT exercise in trained female team-sport players. The findings from this study appear to suggest that 15 min of passive rest may be enough time to recover between exercise modalities in the current concurrent training protocol to enable maximal effort and performance of both exercise modes without evidence of residual fatigue. Concurrent RT and RSA exercise also induced a plasma inflammatory reaction as well as a prompt and significant elevation of cells of the acute phase response, with the exercise order having only a small influence on these responses. In healthy, trained female team-sport players the inflammatory

response to exercise was tightly restricted in magnitude by a balance between pro- and anti-inflammatory cytokines. The changes in the GH↔IGF-1 axis are suggestive of a mild exercise-induced anabolic environment, that was greater following the RT:RSA order. Although this difference was small, it may be physiologically relevant in a chronic training scenario in well trained team-sport athletes. The results of the current study provide an overview of the acute systemic response in the immediate recovery phase following intense concurrent exercise. How this response adapts within a chronic training scenario however, is currently unknown and therefore a training investigation would provide a natural progression to this study. It can be concluded that these bio-markers can be measured in human participants and may be used to gauge physiological stress caused by exercise in an acute setting as they appear to be regulated by exercise intensity and duration. As such, sport scientists and coaches could potentially utilise this information to compare a specific training session with match-play, as well it may be possible to monitor physiological tolerance of an athlete to specific exercise training loads, but further research would be needed in order to validate these possible applications.

CHAPTER 6

The effect of 4 weeks of same or alternating day concurrent resistance and repeated-sprint training on performance in female team-sport athletes

Abstract

Time-motion analysis of field based team-sports suggest that maximal strength and power as well as the ability to sprint repeatedly are important for competitive match performance. As a consequence of the time constraints imposed on team-sport players due to the requirement to improve technical aspects of the game, these two modes of exercise may need to be performed with a single training session. However, there is currently limited research on concurrent resistance (RT) and repeated-sprint ability (RSA) training in female team-sport athletes. Further, how performance adaptation may be affected by same-session RT and RSA training in comparison to single session RT and RSA (24 h apart) concurrent training is currently unknown and may have important practical implications for both athletes and strength and conditioning practitioners. Therefore the aims of the current study were to 1) determine the effectiveness of concurrent RT and RSA training on team-sport specific performance variables and 2) investigate the effect of same day (SDT) and alternating day (ADT) concurrent training on the magnitude of change in performance. Twelve well-trained female team-sport athletes completed speed, RSA, strength and power tests prior to and after a 4 week training intervention. Participants were randomly assigned to either the SDT or ADT training group. Both groups completed the same type and volume of RT and RSA training but the SDT group completed 3 $\times$ same session concurrent resistance and repeated-sprint exercise sessions per week

versus the ADT group who completed 6 × single session concurrent resistance and repeated-sprint exercise sessions per week. No significant training group effects were observed, however significant training effects were observed from pre to post-training for almost all performance tests ($p < 0.05$) with 10 m, 20 m, 30 m maximal sprint, RSA time, squat 1-repetition maximum (1-RM), countermovement jump (CMJ) and static jump (SJ) improving by 4.2 (± 4.4), 3.1 (± 2.9), 3.3 (± 2.3), 1.8 (± 3.4), 24.8 (± 6.1), 6.4 (± 8.2), and 4.9 (± 7.5) % respectively. The current study provides practical evidence that concurrent RT and RSA exercise can be effective for improving team-sport performance variables and performance adaptations irrespective of SDT or ADT structured training sessions in well-trained female athletes.

Introduction

Team-sports are multifaceted requiring an array of fitness qualities including repeated-sprint ability (RSA), maximal strength and explosive power in order to execute the skills required to affect the outcome of the game. The simultaneous development of these fitness qualities presents a significant challenge for strength and conditioning practitioners especially when training for the technical attributes of the game already places considerable demands on training time. Therefore concurrent RSA and lower body resistance training (RT) may be able to provide some answers, allowing for both efficient and effective training strategies particularly during the pre-season. To date, no studies have examined the effect of same day (SDT) or alternating day (ADT) concurrent RSA and RT exercise on lower-body muscular strength, power or RSA in any population.

Previous concurrent training studies have had a predominant focus on the two traditional and most divergent forms of exercise; resistance and endurance training [17, 18, 50, 301, 303]. The majority of studies demonstrate a lower magnitude in strength gains with concurrent resistance and endurance programmes compared with strength training alone [17,18,301], while occasionally aerobic adaptations have been blunted [50]. Others report no interference effect of concurrent training on the development of aerobic fitness and strength gains [46, 303]. While it is difficult to ascertain the causes of interference in concurrent training leading to compromised performance gains, the acute theory proposes that peripheral fatigue induced by the initial exercise may compromise the ability to generate tension and maximal force in the subsequent mode of exercise [52]. Reductions in the ability to generate maximum tension during acute training sessions may lead to a lower magnitude of skeletal muscle adaptation and subsequent performance improvement [322]. Differences in exercise selection, intensity, programme structure and training status likely contribute to the variance in concurrent training outcomes [21], and may be im-

portant factors determining the effectiveness of concurrent training on performance outcomes in team-sport athletes. Due to possible residual fatigue effects on the performance of the second mode of exercise in a concurrent programme it would appear relevant to investigate the effects of SDT or ADT concurrent RT and RSA of the same total volume on strength, power and RSA performance development.

The order effect of RT and endurance exercise in the same session has also been investigated. Cadore et al. [95] found greater maximal strength gains and neuromuscular efficiency when strength training was performed prior to endurance exercise in older men, while 12 weeks of endurance exercise prior to circuit training produced a greater improvement in 4 km running time trial performance than the opposite order in young males [96]. The order of high intensity sprint/endurance and strength training was inconsequential to performance adaptation in professional soccer players following 5 weeks of intervention [30]. A limited number of studies have investigated concurrent repeated-sprint and resistance training specific for team-sport athletes with male participants. These studies provide support for the compatibility of adaptations to these two modes of exercise and thus the inclusion of such exercise programmes for the improvement of team-sport specific performance [22, 30, 323]. There is however, a lack of female specific research in this area and while responses to concurrent training in males may provide some useful information, as female participation in team-sports continues to grow it is essential that female specific research is undertaken.

By assessing the changes in performance measures over the length of a pre-season training phase (4 weeks) to differently structured RT and RSA exercise, coaches and strength and conditioning practitioners can understand the magnitude of changes that can occur in performance in a short period and may be better able to structure effective training programmes. Therefore, the aim of this investigation was to determine the effects of 4 weeks of concurrent RT and RSA training on team-sport specific power and speed performance in female team-sport players. A secondary aim was to determine the optimal structure of such concurrent training by assessing the effects of greater continuous volume in the acute stimulus (SDT) or reducing the chances of peripheral fatigue in the acute stimulus (ADT) on performance adaptation to concurrent RT and RSA.

Methods

Experimental overview

The study took place during the non-competition pre-season build up phase of the year, following an off-season break. After signing informed consent participants were

measured for height, weight and thigh girth and then completed a familiarisation of the experimental exercise protocols to ensure correct technique and maximal effort during testing and training. Two days (48 h) prior to the intervention period participants completed a series of strength, power, speed and aerobic performance tests. The testing began with a 1-repetition maximum (1-RM) back squat test for assessment of maximal strength. Following 10 min of rest, participants completed a counter movement jump (CMJ) and static jump (SJ) for tests of peak power production. After 15 min rest, participants completed a 30 m maximal sprint test followed by a graded exercise test (GXT) for $\dot{V}O_2$peak. Two days (48 h) after initial performance testing, all participants completed an acute concurrent RT and RSA protocol consisting of 6 × 6 sets of back squat at 80 % 1-RM followed 15 min later by 6 × 4 sets of maximal 20 m shuttle sprints. Two days (48 h) after completion of the acute concurrent exercise protocol, participants were randomly divided into two training groups (SDT and ADT training groups) and began a 4 week training intervention (outlined below). Thirty six-48 h following the last training session participants completed all performance tests again, and 48 h following performance testing participants completed the acute concurrent RT and RSA protocol again (Figure 6.1.).

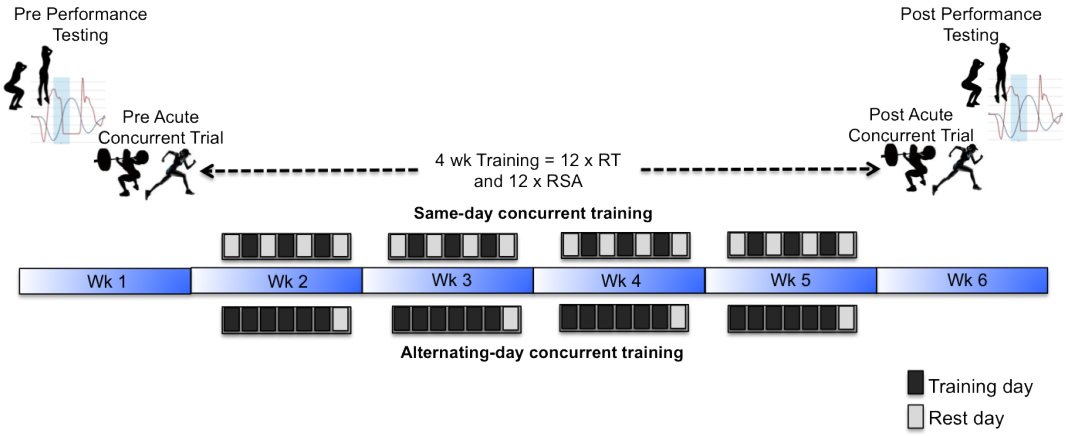


Figure 6.1: Overview of experimental protocol.

Participants

Twelve well-trained female team-sport (soccer, hockey, netball, touch) athletes aged 18-30 y involved in regular team-sport competition for a minimum of 5 years were recruited for this study. Participants were top level club or provincial representative players training within the team structure at least 3 times a week during in-season and had some history of lower body resistance training. Participants were required to have a $\dot{V}O_2$peak above 40 mL·kg^{-1}·min^{-1} and a maximal squat equivalent to 85

% body weight or greater. Participants were randomly assigned to either the SDT or the ADT training group (Table 6.1). Participants were informed of the possible risks and discomforts of participating in the proposed research prior to providing written informed consent. This study was approved by the Auckland University of Technology Ethics Committee and conformed to standards for the use of human subjects in research as outlined in the Declaration of Helsinki.

Table 6.1: Participant characteristics.

Descriptive	SDT	ADT
Age (y)	21.0 ± 3.0	25.4 ± 4.1
Body mass (kg)	68.2 ± 6.8	62.2 ± 2.6
Height (cm)	172.2 ± 7.0	167.3 ± 4.3
Relative Squat 1-RM	1.0 ± 0.1	1.02 ± 0.2
$\dot{V}O_2$peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	44.2 ± 4.0	44.7 ± 4.0

Data presented as means ± SD.

Participant controls

Menstrual and contraceptive controls

To control for menstrual status, all participants were required to be taking the active pills of a combined monophasic oral contraceptive pill (Levonorgestrel 0.15 mg and Ethinyloestradiol 0.03 mg) for the entire duration of the study (i.e. no withdrawal period during the study). Participants that had been diagnosed with a menstrual disorder that is known to increase circulating androgen levels were excluded from participating in the current research.

Dietary and exercise controls

Athletes were instructed that for the duration of the study they were not to perform any other exercise than that prescribed as either part of the experimental training programme or the performance testing. Participants were required to keep a 48 h food diary on the day of and prior to testing and were asked to maintain the same diet prior to each experimental trial and performance testing. Participants were provided with a standardised meal (∼51 KJ energy per kg of body mass; ∼78 % carbohydrate (CHO), ∼8 % Fat, ∼14 % Protein) to be eaten 4 h (13:00) prior to experimental acute trials and participants were also supplied with 3 nutritionally identical breakfast options (∼27 KJ energy per kg of body mass; ∼85 %CHO, ∼5 % Fat, ∼10 % Protein) to be eaten on the morning of the trial. No caffeine or alcohol

was permitted to be consumed within 24 hours of experimental trials and performance testing. During the training period participants were instructed to consume no sports drink during training (water was allowed) and were asked to refrain from eating within 90 minutes prior to and post-training to reduce dietary and nutrition effects on the training adaptation. All experimental trials took place between 16:30 and 22:00 h (exercise between 17:00 and 18:30 h). Therefore participants were required to complete all training sessions within the hours of 17:00 and 20:00 h. All performance testing was conducted at the same time of day for each participant.

Graded exercise test

Participants completed a graded exercise test to volitional exhaustion on a motor-driven treadmill (Saturn 4.0, h/p/ Cosmos Sports and Medical GmbH, Nussdorf-Traunstein, Germany) pre and post-training to determine $\dot{V}O_2$peak. The test began with at 8 km·h^{-1} at a 1 % gradient followed by an increase of speed to 10 km·h^{-1} after 2.5 min. Thereafter the treadmill speed was increased by 1 km·h^{-1} every 2.5 min until volitional exhaustion was achieved. Determination that $\dot{V}O_2$peak was reached was confirmed with a respiratory exchange ratio above 1.10, heart rate (HR) within 10 beats of predicted maximal HR and a rating of perceived exertion (RPE) of 20. Expired air was measured continuously for concentrations of oxygen and carbon dioxide using a calibrated PARVO metabolic cart (Parvo Trueone, Sandy, Utah). The metabolic cart was calibrated prior to each test to ensure accuracy of measurement. Peak oxygen consumption was defined as the highest value achieved during the graded exercise test.

Anthropometric measures

Participants were measured for height and weight to the nearest 0.5 cm and 0.1 kg, respectively. Thigh girth was measured as per International Society for the Advancement of Kinanthropometry (ISAK) methods. To increase the reliability of this measure the same researcher performed all thigh girth measurements.

1-repetition maximum test

To determine 1-RM, participants performed squats for 8-10 repetitions at 50 % of their estimated 1-RM followed by another set of 2-5 repetitions at 85 % of estimated 1-RM. Subsequently, 4-5 1-repetition trials were used to determine the 1-RM [284]. A lift was considered successful once participants had descended to the point where the tops of the quadriceps were parallel with the floor. The reliability of this test in our laboratory is high with intraclass correlation coefficient (ICC) = 0.97 and

coefficient of variation (CV) = 2.5 %. The load achieved during the 1-RM was subsequently used to calculate the load to be lifted during the acute experimental concurrent exercise trial and during training (80 % of 1-RM).

Jump testing

CMJ and SJ were performed with participants standing on a force plate (400 Series Performance Force Plate, Fitness Technology, Australia) which was interfaced with software (Ballistic Measurement System, Fitness Technology, Australia) that allowed for direct measurement of force-time characteristics as outlined by McGuigan et al. [307]. Participants were instructed to keep their hands on their hips at all times throughout the movement. Participants began the CMJ by initiating a self-selected counter movement then explosively jumping as high possible in the opposite direction. The SJ began from a self-selected semi squatting position held for 3 seconds before subsequent upwards movement and jumping as high possible. Participants performed 3 trials of each jump, and the jumps that recorded the highest peak power were used for subsequent analysis [307]. For all variables associated with these tests the ICC is > 0.98 and CV's < 4 %. The eccentric utilisation ratio (EUR) was calculated by dividing relative power (relative to body weight) achieved in the CMJ by the relative power achieved in the SJ [307].

Sprint testing

Maximal sprint times for 10 m, 20 m and 30 m were measured using an indoor athletics track with electronic timing gates (Speed Light Sports timing system, SWIFT, NSW). Participants completed 10 min of individual dynamic warm-up including progressive accelerations and dynamic stretching. Participants then completed 3 trials separated by 3 min of rest, each trial began in the participants own time, 30 cm behind the starting timing gate from a standing start. The fastest trial was used for subsequent analysis. The reliability for this test in our laboratory is high: ICC = 0.95 and CV < 2 %.

Acute concurrent resistance and repeated-sprint protocol

The acute concurrent RT and RSA protocol consisted of ∼20 mins of back squat exercise and ∼17 min of RSA exercise separated by 15 mins of passive rest (Figure 6.2).

Squat protocol

Warm-up: Prior to beginning the squat protocol either when performed in isolation (ADT) or within a concurrent exercise session (acute trial and SDT) participants

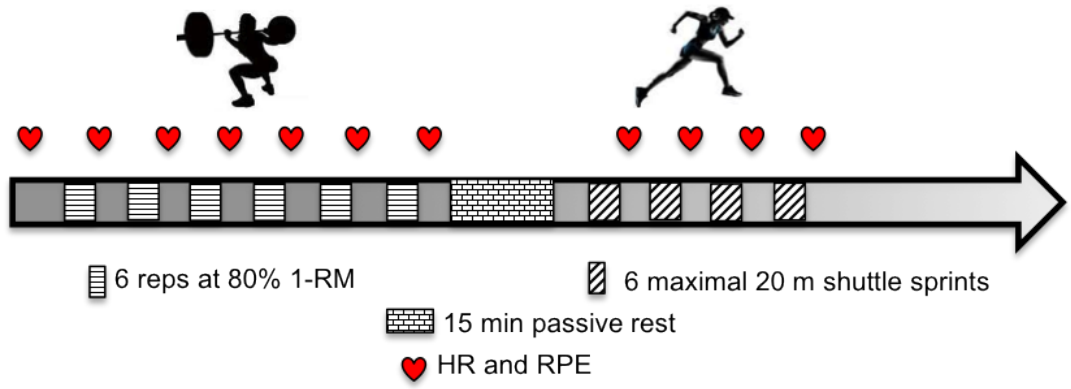


Figure 6.2: Overview of acute concurrent RT and RSA protocol.

were required to complete 1 set of 6 repetitions with the bar only (20 kg) followed by 1 set of 6 repetitions at ∼50 % of their experimental load, followed by another set of 6 repetitions at ∼70 % of their experimental load.

The RT protocol consisted of 6 sets of 6 repetitions with 80 % of 1-RM for the back squat exercise with 3 min of passive rest between sets.

Repeated-sprint protocol

Warm-up: Prior to beginning the repeated-sprint protocol participants were required to jog 6 × 20 m lengths of the shuttle protocol turning as they would for the actual trial. Once this was completed participants were instructed to perform 1 warm-up sprint set consisting of 6 progressive sprints; 2 shuttle sprints at ∼60 %, ∼70 % and ∼80 % of maximal speed in preparation for the actual exercise trial. Participants were allowed 3 min rest between the end of the warm-up and the start of the experimental repeated-sprint protocol.

The repeated-sprint protocol consisted of 4 sprint sets with each sprint set consisting of 6 × 20 m maximal running shuttle sprints. A line was placed at 10 m, allowing participants to sprint and place both feet over the line before turning and sprinting back to the start line. Participants were required to sprint every 20 s during each sprint set. Participants had 3 min passive rest between sets and were allowed to consume water ad libitum. Electronic timing gates (Speed Light Sports timing system, SWIFT, NSW) were placed at the start/finish line to measure velocity and time (s) for each sprint. A relative (percentage decrement over the repeated efforts) repeated-sprint fatigue score was calculated for each of the 6 × 20 m sprint sets, calculated using the percentage decrement method [306]. The reliability for total sprint time in our laboratory is high with ICC = 0.95 and CV = 1.7 % however, reliability for total sprint decrement across all sets was less reliable with ICC = 0.79

and CV = 14.5 %. To increase the reliability participants were familiarised with the sprint protocol prior to beginning the study.

Heart rate and ratings of perceived exertion

During the RT and RSA protocol HR and RPE were recorded immediately post each sprint set. HR was measured using a Polar RS800 watch, and the 15 point (6-20) Borg Scale [287] was used.

Training intervention

The SDT group completed 12 training sessions in total (3 × training sessions per week for 4 weeks) identical to the acute concurrent RT and RSA protocol above with at least 48 h between training sessions equating to 3 training days per week and 4 non-exercise days per week. The ADT group completed 24 sessions in total (6 sessions per week for 4 weeks) of alternating (separated by 24 h) single mode RT exercise or RSA exercise with 24 h between training sessions equating to 6 consecutive training days per week and 1 non-exercise day per week (same total volume of training for each group) (Figure 6.3). Participants were instructed that recovery from exercise should consist of passive rest only and that no other recovery modalities were to be used (i.e. the use of massage or anti-inflammatory products).

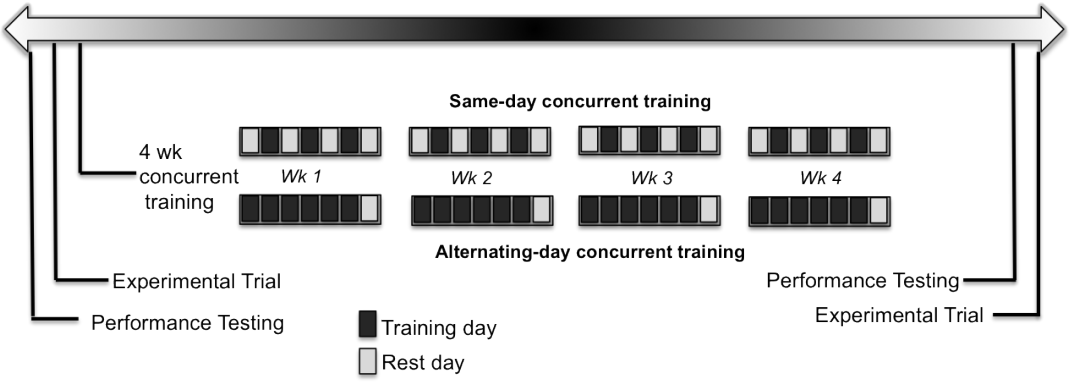


Figure 6.3: Overview of 4 week training intervention.

Statistical analysis

Independent samples t-tests were used to compare pre-training descriptive data for the 2 training groups. An ANCOVA was used to determine differences between groups from pre- to post-training, using the pre-training measure as a covariate to adjust for baseline differences between groups. Where no statistical differences were

observed between the two groups pre and post measures, groups were pooled and paired t tests were performed on the data ($n = 12$) to compare changes between pre and post-training measures. Significance was set at an alpha level of 0.05, with data presented as mean ± standard deviation (SD). Cohen's d effect sizes (ES) were used to determine practical magnitude of training effect between the mean of the variables over time. ES of > 0.2, > 0.6, > 1.2 and > 2.0 were interpreted as small, moderate, large and very large, respectively [324]. A z-score for each performance measure for each athlete was calculated as a measure of the athletes performance within the population using the formula prescribed in McGuigan et al. [325] ((athlete score-benchmark)/benchmark standard deviation) where the benchmark scores have been developed on previous testing data within a similar population.

Results

There were no differences between groups pre-training for any variables. All training sessions, 12 for the SDT training group and 24 for the ADT training group were performed by all participants (100 % compliance).

Heart rate and rating of perceived exertion

Results for HR and RPE are presented in Table 6.2. There were no effects for training group on HR or RPE responses. Average HR achieved during the RT and RSA protocols did not differ pre to post-training ($p = 0.11$ and $p = 0.40$). Average HR was significantly lower during RT than RSA ($p < 0.01$). Average RPE achieved during RT was lower post-training ($p < 0.01$), but average RPE achieved during RSA was unchanged with training ($p = 0.48$). Average RPE achieved during the RT protocol was significantly lower than RPE achieved during the RSA protocol both pre and post-training ($p < 0.01$).

Table 6.2: Acute RPE and HR responses following each set of the RT and RSA exercise pre and post 4 weeks of concurrent RT:RSA training in female team-sport athletes.

	Ex. mode	Time	Rest	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Average
RPE	RT	Pre		12 (2)	13 (2)	13 (1)	14 (1)	14 (2)	15 (2)	13 (1)$^{\#}$
	RT	Post		11 (2)	12 (2)	12 (2)	12 (1)	13 (2)	13 (2)	12 (1)$^{\#}$†
	RSA	Pre		15 (2)	16 (2)	17 (2)	18 (2)			16 (1)
	RSA	Post		15 (2)	17 (2)	18 (1)	19 (1)			17 (2)
HR ($beats \cdot min^{-1}$)	RT	Pre	76 (16)	123 (22)	131 (19)	137 (19)	139 (19)	139 (18)	143 (17)*	135 (21)$^{\#}$
	RT	Post	70 (9)	125 (25)	129 (18)	132 (16)	129 (15)	130 (15)	131 (15)	130 (18)$^{\#}$
	RSA	Pre	76 (16)	180 (9)	184 (9)	184 (8)	183 (11)			183 (8)
	RSA	Post	70 (9)	180 (11)	184 (10)	185 (9)	185 (10)			180 (17)

Data presented as mean (SD), $n = 12$. $^{\#}$Significantly different from RSA, $p < 0.05$.
†Significantly different from pre-training, $p < 0.05$

Same day or alternating day concurrent training effects

There were no statistically significant differences in the changes for any of the measured performance variables (pre to post-training) between the two training groups. However there was a trend for the CMJ to improve by a greater extent in the ADT group (p = 0.070) and for the SJ to improve by a greater extent in the SDT group ($p = 0.052$). ES indicate that practically, SDT was more effective at increasing SJ performance (SDT 0.71 vs. ADT 0.35; Figure 6.4 Table 6.3) and ADT training was more effective for increasing CMJ performance (SDT 0.37 vs. ADT 0.93; Figure 6.4 and Table 6.3).

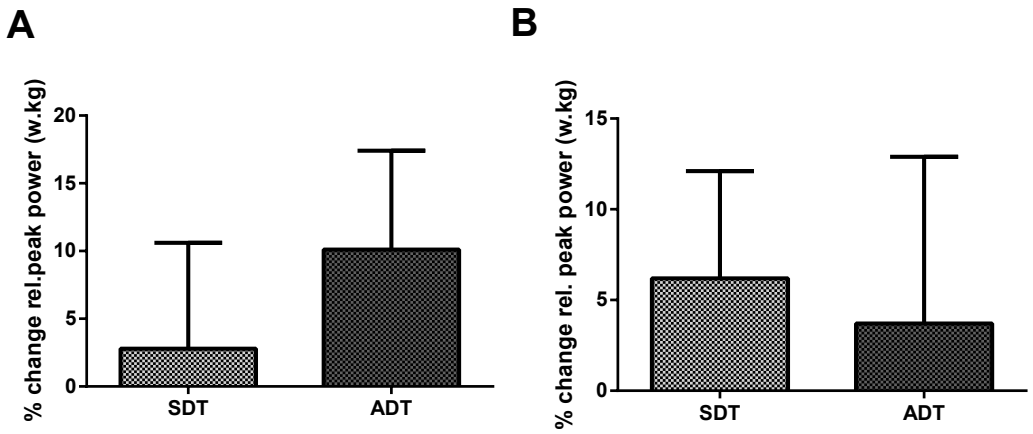


Figure 6.4: Percentage change pre to post 4 weeks of concurrent training for (A) CMJ and (B) SJ for SDT (n =6) and ADT (n =6) training groups.

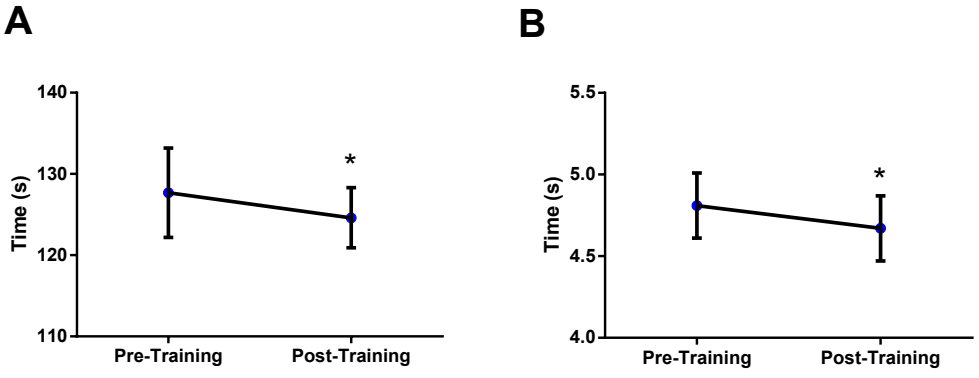


Figure 6.5: (A) Total time for all sprints during RSA protocol (4 × 6 20 m shuttle sprints) and (B) Maximal 30 m sprint time pre and post 4 weeks of concurrent RT and RSA training in female team-sport athletes. As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. n = 12) for pre to post-training are presented. *Significantly different from pre-training, $p < 0.05$.

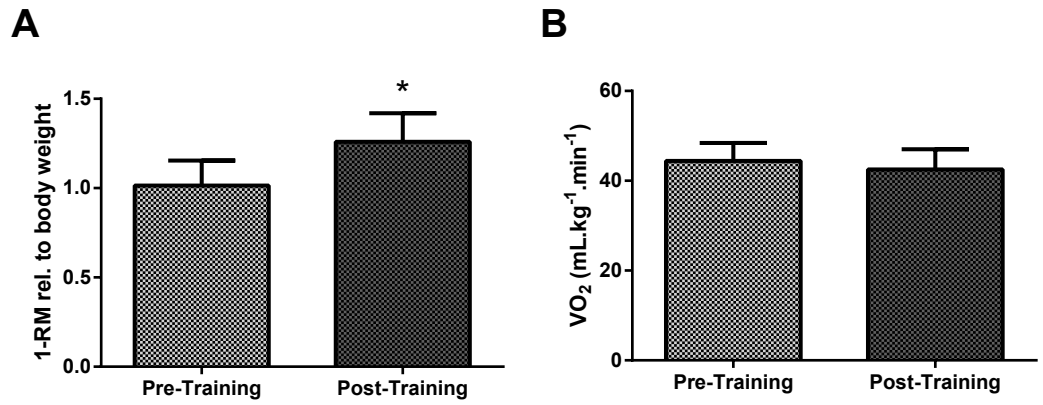


Figure 6.6: (A) Back squat 1-RM (relative to body weight) and (B) $\dot{V}O_2$ peak ($mL{\cdot}kg^{-1}{\cdot}min^{-1}$) pre and post 4 weeks of concurrent RT and RSA training in female team-sport athletes. As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. n = 12) for pre to post-training are presented. *Significantly different from pre-training, $p < 0.05$.

Training effects

There were significant effects for training irrespective of training group (i.e., pre to post-training) for CMJ, SJ, 1-RM, 10 m, 20 m and 30 m maximal sprint times and the RSA total sprint time (all measures $p < 0.05$). Training did not significantly alter $\dot{V}O_2$ peak, RSA average sprint decrement per set or thigh girth ($p > 0.05$). These data as well as the associated percentage change in performance and ES are presented in Tables 6.3 and 6.4 as well as Figures 6.5, 6.6 and 6.7. The ES for mean change from pre to post-training ranged from small to large.

Table 6.3: Strength and power performance pre and post 4 weeks of concurrent RT and RSA training in female team-sport athletes.

Test	Group	Pre	Post	% Change	p value	ES Pre-Post
CMJ (watts/kg)	SDT	38.2 (3.4)	39.3 (2.7)	2.8 (7.8)		0.37
	ADT	39.9 (4.7)	44.6 (5.4)	10.1 (7.3)		0.93
	Pooled	39.0 (4.0)	41.9 (4.9)	6.4 (8.2)	0.02	0.65
SJ (watts/kg)	SDT	36.9 (3.4)	39.4 (3.7)	6.2 (5.9)		0.71
	ADT	40.3 (3.8)	42.4 (7.7)	3.7 (9.2)		0.35
	Pooled	38.6 (3.9)	40.9 (6.0)	4.9 (7.5)	0.04	0.46
1-RM (rel. B/W)	SDT	1.0 (0.13)	1.25 (0.15)	24.9 (5.9)		1.78
	ADT	1.02 (0.15)	1.27 (0.18)	24.7 (6.9)		1.51
	Pooled	1.01 (0.14)	1.26 (0.16)	24.8 (6.1)	0.00	1.67
EUR	SDT	1.04 (0.10)	1.00 (0.05)	-3.87 (8.36)		0.46
	ADT	0.99 (0.08)	1.06 (0.07)	5.99 (12.61)		0.93
	Pooled	1.02 (0.09)	1.03 (0.07)	1.06 (11.43)	0.63	0.19
Thigh Girth	SDT	53.79 (3.79)	54.63 (3.89)	1.55 (1.55)		0.22
	ADT	53.97 (3.17)	53.95 (2.48)	0.03 (1.88)		0.01
	Pooled	53.88 (3.32)	54.29 (3.13)	0.79 (1.84)	0.177	0.13

Data presented as mean (SD) including relative change (%) pre to post training for each variable, p value and effect size (ES), $n = 12$. SDT = same day training group. ADT = alternating day training group. CMJ = countermovent jump. SJ = static jump. 1-RM = 1-repetition maximum. EUR = eccentric utilisation ratio.

Table 6.4: Maximal speed, RSA, and $\dot{V}O_2$peak performance data pre and post 4 weeks of concurrent RT and RSA training in female team-sport athletes.

Test	Group	Pre	Post	% Change	p value	ES Pre-Post
10 m Sprint (s)	SDT	1.97 (0.11)	1.87 (0.10)	-5.6 (4.9)		0.95
	ADT	1.95 (0.06)	1.90 (0.09)	-2.9 (3.7)		0.65
	Pooled	1.97 (0.09)	1.89 (0.09)	-4.2 (4.4)	0.005	0.90
20 m Sprint (s)	SDT	3.42 (0.16)	3.27 (0.16)	-4.4 (3.1)		0.94
	ADT	3.40 (0.11)	3.34 (0.15)	-1.8 (2.2)		0.46
	Pooled	3.41 (0.13)	3.31 (0.15)	-3.1 (2.9)	0.002	0.71
30 m Sprint (s)	SDT	4.81 (0.21)	4.64 (0.22)	-4.5 (2.3)		0.76
	ADT	4.81 (0.13)	4.70 (0.2)	-2.4 (1.9)		0.65
	Pooled	4.81 (0.2)	4.67 (0.2)	-3.3 (2.3)	0.001	0.76
RSA (t) (Total (s)	SDT	125.60 (5.1)	123.36 (3.8)	-1.8 (2.8)		0.50
	ADT	129.80 (5.4)	125.90 (3.4)	-3.07 (1.6)		0.90
	Pooled	127.70 (5.5)	124.60 (3.7)	-1.8 (3.4)	0.004	0.77
RSA (% dec) (Av % dec/bout)	SDT	3.3 (1.5)	2.7 (1.5)	-39.2 (70.3)		0.41
	ADT	3.7 (0.8)	3.4 (1.2)	-23.0 (46.2)		0.37
	Pooled	3.2 (1.0)	3.0 (1.1)	-30.8 (55.8)	0.264	0.60
$\dot{V}O_2$ peak ($mL{\cdot}kg^{-1}{\cdot}min^{-1}$)	SDT	44.2 (4.0)	42.3 (4.1)	-2.7 (0.04)		0.46
	ADT	44.7 (4.0)	42.7 (5.1)	-5.1 (0.06)		0.44
	Pooled	44.4 (4.0)	42.5 (4.5)	-4.3 (5.3)	0.217	0.44

Data presented as mean (SD) including relative change (%) pre to post training for each variable, p value and effect size (ES), $n = 12$. SDT = same day training group. ADT = alternating day training group. RSA = repeated-sprint ability. % dec = percentage decrement.

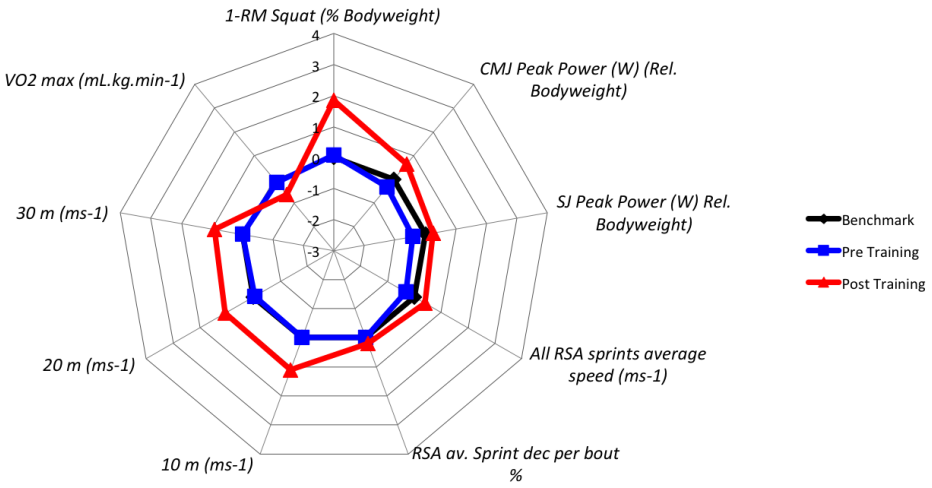


Figure 6.7: Radar plot representing overall (n= 12) pre and post 4 weeks of concurrent RT and RSA training z-scores for performance measures in female team-sport athletes.

Discussion

The current study is the first to investigate the effects of concurrently training RT and RSA exercise in well trained female team-sport athletes as well as being the first to investigate the effects of SDT versus ADT concurrent RT and RSA training on team-sport performance variables. The results demonstrate significant beneficial effects of concurrent RT and RSA training, both within the same session and on alternating days on a range performance variables, including sprinting, jumping, RSA and maximum leg strength.

It has been shown that RSA can be an important fitness component governing selection for team-sport athletes [299]. In addition, improving the power to weight ratio of team-sport players has been shown to be important for enhancing performance [122]. Wisløff et al. [300] found a positive relationship between strength, endurance capacity and soccer match performance demonstrating that improving pre-season endurance and strength variables enhanced subsequent match results during the season. Many reports suggest that concurrent strength and endurance training inhibits strength development compared with strength training alone [17,18,301]. However, previous studies have used an RSA or high-intensity interval training (HIT) and RT exercise program instead of the traditional endurance (sub-maximal) and resistance exercise profile to improve the specificity of concurrent training in male team-sport athletes [22, 30, 323] and found similar positive improvements in performance to that of the current study.

An 8 week intervention in which male soccer players completed 2 strength and high-intensity interval training (16 × 15 s sprints at 120 % maximum aerobic

speed with 15 s rest) sessions in addition to regular soccer training performed by the control group produced significant improvements in CMJ height, 10-30 m sprint, Yo Yo Intermittent Recovery Test 1 (YYIRT1), and maximum aerobic speed compared with control [22]. These findings suggest that the longer duration sprints used in this intervention had a positive effect on aerobic capacity, in contrast to the shorter duration sprints used in the current investigation. A second study employing an 8 week intervention of concurrent half-squat and aerobic interval training (4 × 4 min intervals at 90-95 % max HR) alongside regular soccer training in elite male soccer players found significant improvements in half-squat 1-RM, $\dot{V}O_2$ max, 10 m sprint and CMJ performance [323] comparable to the improvements reported in trained female athletes in the current study. However, both of these previous investigations did not measure RSA. Further, a recent study that investigated the order effect of concurrent high-intensity training (various sprint protocols with and without the ball) and strength training (various upper and lower body strength exercises) over a 5 week pre-season period in professional male soccer players found no effects for intra-session exercise order (RT:HIT vs. HIT:RT) [30]. They did however, observe positive training effects irrespective of exercise order for all performance measures, including 10 m sprint, 6 × 30 m repeated sprint, CMJ, and maximal leg strength comparable to the current intervention as well as increases in agility and YYIRT1 test performances. The findings from all 3 studies are similar to the findings of the current study suggesting concurrent RT and RSA exercise may be a positive time-efficient addition to team-sport training programmes, and that total training volume, not intra-session exercise order, nor training structure (SDT vs. ADT) is more important. It does however, appear that longer duration sprints may be required in order to improve aerobic capacity. Interestingly, the current study found large improvements in team-sport specific performance variables in only 4 weeks of concurrent RT and RSA training and is also the first study to provide a practical and successful concurrent RT and RSA training protocol in trained female team-sport athletes.

As the development of RSA, power, strength and endurance capacities are important for team-sport performance [11, 121, 122, 299], concurrent training in some form is likely to be undertaken as part of pre-season and in-season training for team-sport athletes. Therefore, a focus of the current study was to determine if there may be differences in performance improvement following RT and RSA exercise of the same total volume performed either within the same session separated by 15 min (SDT) or on consecutive days 24 h apart (ADT). The findings suggest comparable improvements in performance between the SDT and ADT concurrent training groups. However, ES scores suggest there may be some practical differences in SJ and CMJ improvements between the two training groups, yet there was some inter-subject variability in the change from pre to post training in these performance

measures. Though, together with the differences in the EUR (although not significant) pre to post-training in the SDT group (pre 1.04 ± 0.10 - post 1.00 ± 0.05) and ADT group (pre 0.99 ± 0.08 - post 1.06 ± 0.07), may suggest divergent effects of the differently structured training programmes on the ability to produce explosive power using the stretch-shortening cycle and may provide an interesting area for future work.

It was demonstrated in the current study that the RT and RSA training as part of a pre-season training programme for female team-sport athletes was effective at increasing power and strength based variables of team-sport performance, including, 10 m, 20 m and 30 m sprint time, CMJ and SJ. The gains in strength and power were similar to those reported in previous studies employing strength training in team-sport male and female athletes [27, 326, 327]. Interestingly though, in these studies the RT exercise was performed in isolation, so it appears that performing RSA after lower body RT exercise may not inhibit or hinder strength and power performance adaptations in female team-sport athletes over 4 weeks. The significant training effects seen in 1-RM squat (∼20 %) in the current study likely contributed to the increases in sprint performance also seen as it has previously been shown that stronger team-sport athletes also produce greater sprint performances [16, 122, 277, 328] with the strongest strength predictor of sprint speed occurring when strength is assessed using free weights (-0.64 relative, 0.94 absolute) [329]. This provides support for lower body strength training to be included in the programme design for team-sport athletes.

RSA total sprint time was improved by an average of 1.8 % following concurrent RT and RSA exercise in the current study, which is similar to the improvement in RSA total sprint time reported by McGawley et al. [30] in professional soccer players following 5 weeks combined HIT and strength training. The training protocols in the current study did not however have a positive impact on $\dot{V}O_2$peak which may have contributed to why the fatigue index during the RSA protocol was not improved significantly within the current study. This result is a contrast to the significant improvements in YYIRT Level 2, and RSA % decrement reported by McGawley et al. [30] who employed varied sprint interval training from shorter distance repeated-sprints to intervals of 4 min on/3 min active rest at 90-95 % $\dot{V}O_2$max and may explain the differences in aerobic performance between the two studies. However, in a homogenous group of elite female team-sport athletes $\dot{V}O_2$peak was not shown to be a strong predictor of RSA during a 5 × 6 s cycle test [330] and a study carried out in 42 male footballers found neither high-intensity intervals (4 × 4 min running at 90-95 % HR max) nor RSA training (3 × 6 maximal shuttle sprints of 40 m) was able to improve sprint decrement after 7 weeks of training, but RSA training did improve RSA time [331]. Therefore, it is unclear whether improvements in maxi-

mal aerobic capacity would have affected sprint decrement in the female participants of the current study.

While no statistical difference of concurrent training structure (SDT versus ADT) was evident following 4 weeks of training, it may be that this training period and 12 SDT or 24 ADT RT and RSA concurrent training sessions was not long enough to detect a difference in training stimulus on performance adaptation. A longer duration training period may have elucidated some significant differences in performance. Studies employing a longer training duration of 10-21 weeks have reported interference of concurrent endurance and strength training on strength and explosive power development [17, 18, 301]. Further, even though the participants in this study were representative team-sport athletes and well-trained, the training in the current study was undertaken in the pre-season period, and it may be expected that either training programme would improve performance following a short period of detraining. Further research may be required to determine if performance remains similar between the training groups over a prolonged duration if an extended training period ($>$ 4 weeks) is desired.

Conclusion

The current study provided practical evidence for the inclusion of concurrent RT and RSA exercise training during the pre-season in female team-sport athletes in order to improve RSA, maximal sprint and maximal strength and power parameters that may improve preparedness for in-season training and improve in-season match performance. The training strategy is effective when performed within the same session or on alternating days suggesting it is not detrimental to performance to train both RSA and lower body RT training in the same session if required or if time constraints exist. The strength and conditioning practitioner can be confident that whether performing RT exercise and RSA exercise in the same session or on alternating days, similar training induced performance adaptations will be achieved in well trained female team-sport athletes.

CHAPTER 7

Cytokine, hormone and molecular responses to 4 weeks of concurrent resistance and repeated-sprint training in female team-sport athletes

Abstract

The current study took a novel approach to the largely researched model of concurrent training by altering the modes of exercise for the requirements of team-sport athletes. The acute inflammatory, hormonal and molecular response to concurrent resistance (RT - 6 x 6 squats at 80 % 1-RM) and repeated-sprint (RSA - 4 x 6 20 m shuttle maximal sprints) exercise was investigated in the 180 min post-exercise recovery period before and after 4 weeks of same day (SDT, $n = 6$) or alternating day (ADT $n = 6$) concurrent training in female athletes. Muscle biopsies (vastus lateralis) were taken at pre-exercise and 60 min and 180 min post-exercise. Blood samples were drawn at pre-exercise, immediately post-exercise (0 min), 15 min, 60 min and 180 min post acute concurrent RT and RSA exercise pre and post 4 weeks of training. No training group (SDT vs. ADT) differences were observed pre-post-training in any measures. Plasma IL-6 was elevated at 60 min post-exercise and was accompanied by increases in a number of circulating pro (IL-2, IL-12, TNF-α, IL-8) and anti-inflammatory (IL-4, IL-10, IL-5, IL-7, GM-CSF) cytokines within 180 min of exercise ($p < 0.05$). Skeletal muscle TNF-α and IL-8 protein concentration were significantly increased at 180 min ($p < 0.05$) and both 60 and 180 min respectively, regardless of training status ($p < 0.05$). Interestingly, no changes in IL-6 protein concentration in skeletal muscle were observed. Circulating and local cytokine responses to acute concurrent exercise were not significantly altered by 4

weeks concurrent RT and RSA training. 4E-BP1 protein was significantly depressed at both 60 min and 180 min post-exercise ($p < 0.05$) while p70S6K activity was modestly upregulated at 60 min post-exercise ($p < 0.05$) irrespective of training status. Cortisol and GH were significantly elevated within the 15 min following exercise irrespective of training status ($p < 0.05$) but cortisol response was lowered following training ($p < 0.05$). Glucagon, glucose, insulin and C-peptide were elevated in the 15 min following exercise ($p < 0.05$) but only the plasma glucose response to acute exercise was lowered by training. Squat 1-RM and 30 m sprint time were significantly improved by the same magnitude following both SDT and ADT training ($p < 0.05$). The structure of concurrent RT and RSA training had no effect on the acute physiological response to same session RT and RSA concurrent exercise before and after 4 weeks of training. Training status (pre-post-training) had minimal effect on the acute physiological responses to exercise while importantly, significant improvements in strength and power were still observed.

Introduction

The physiological and performance characteristics of a number of field based team-sports suggest that resistance (RT) and repeated-sprint ability (RSA) training may be important for the improvement and preparedness for competitive match performance [122, 299, 300]. Further, due to the time required to work on the technical aspects of the game, these two modes of exercise may need to be performed within the same session, a phenomenon termed concurrent training. There is however, little current literature regarding the local and systemic physiological, inflammatory and molecular response to both acute and chronic concurrent RT and RSA training in a competitive female team-sport population.

Cytokines are potent intracellular signalling proteins that are thought to regulate the inflammatory response during exercise both locally and systemically [34]. Interleukin (IL)-6 has been termed inflammation responsive or immuno-modulator as it is typically the first cytokine present in the circulation during exercise and its appearance precedes that of other cytokines [159]. IL-6 is thought to act indirectly to restrict inflammation by stimulating the production of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (IL-1ra) [183] and has also been shown to have an inhibitory effect on TNF-α and IL-1β production [332]. It has previously been shown that an elite female soccer match [145], and acute combined RT and RSA (Chapter 5) training can trigger a systemic inflammatory response inducing the elevation of circulating cytokines immediately post-exercise and 2 h in to recovery in trained females. In addition to the role of IL-6 as an inflammatory mediator during exercise, studies have focused on the metabolic effects of this cy-

tokine [35, 36, 170, 172]. IL-6 has consistently been found to be expressed in human skeletal muscle [146, 279]. It has been demonstrated that muscle contraction itself rapidly increases gene expression of IL-6 at the onset of exercise, and IL-6 protein is released from the muscle in to the bloodstream during exercise [136]. The expression and protein release of IL-6 during exercise appears to be heightened when muscle glycogen stores are low [146, 279]. Further, the production of and release of IL-6 by the contracting muscles is said to account for the increases in plasma concentration seen during exercise [136].

Protein or mRNA expression of inflammatory mediators including IL-6, IL-15, IL-4 and IL-8 have been shown to be elevated within contracting skeletal muscle fibres during and post-exercise [155,215,279]. These mediators are increasingly being found to have diverse roles, and as well as contributing to the local inflammatory response, may participate in the repair and adaptation of muscle tissue to exercise by influencing proliferation and myogenic differentiation of satellite cells and promoting angiogenesis [177, 193].

Adaptations within the skeletal muscle at the molecular level are highly specific to the mode of exercise [58]. Skeletal muscle can increase myofibrillar proteins resulting in increased size and strength in response to heavy overload exercise and can increase oxidative capacity by increasing mitochondrial content, lowering glycogen and blood glucose reliance and reduced fatigue in response to prolonged sub-maximal exercise [57, 234, 333]. The AMP-activated protein kinase (AMPK)-peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) pathway is frequently implicated in the adaptive responses to endurance training, while the Akt-Protein Kinase B (Akt)/mammalian target of rapamycin (mTOR) signalling pathway is linked to increased protein synthesis and hypertrophy following resistance exercise [58]. However, the simultaneous activation of divergent pathways during traditional concurrent training (endurance and resistance) has tended to produce interference between training induced adaptations [71, 334]. The molecular response to concurrent RT and RSA training with a similar work:rest ratio is currently unknown.

To date, the majority of studies examining the exercise training effects on cytokine regulation have focused principally on circulating cytokine concentrations or gene expression within skeletal muscle. A number of these studies have focused on the effects of chronic exercise training on resting cytokine levels and therefore there is less research on the acute response post-exercise. How this post-exercise response differs after a period of training is also relatively unknown. The paralleled local and systemic responses have not been thoroughly investigated following exercise in the same participants. Further, the molecular response to concurrent maximal repeated running sprints and lower body resistance exercise is unknown. The aim of this

study was to provide a detailed exploration of inflammatory, hormonal and molecular responses to concurrent RT and RSA training within the context of adaptation for subsequent improvement in performance in female athletes.

Methods

Participants

Twelve female team-sport (soccer, hockey, netball, touch football) athletes aged between 18-30 y were recruited for this study to take place during the pre-season period (Table 7.1). Team-sport athlete was defined as completing at least two regular team-sport trainings and one game per week and competing at top club or provincial level during the in-season and had some history of lower body resistance training. All participants were fully informed of the scientific interest and rationale for the study as well as the methodological protocols and expectations of participants before providing written informed consent. This study was approved by the Auckland University of Technology Ethics Committee and conformed to standards for the use of human subjects in research as outlined in the Declaration of Helsinki.

Table 7.1: Participant characteristics.

Descriptive	SDT	ADT
Age (y)	21.0 ± 3.0	25.4 ± 4.1
Body mass (kg)	68.2 ± 6.8	62.2 ± 2.6
Height (cm)	172 ± 7	167 ± 4
Relative Squat 1-RM	1.00 ± 0.10	1.02 ± 0.20
$\dot{V}O_2$peak(mL·kg^{-1}·min^{-1})	44.2 ± 4.0	44.7 ± 4.0

Data presented as means ± SD.

Experimental protocol

Following study recruitment all participants were randomly divided in to 2 training groups and underwent anthropometric measures (height, mass, and thigh girth). During this initial visit, participants also completed familiarisation procedures, where all exercise testing and experimental trial procedures were explained and practised. During this initial visit participants performed one repetition-maximum (1-RM) strength testing for the back squat exercise, a 30 m maximal linear sprint test and an incremental graded exercise test (GXT) on an electronically braked treadmill to measure $\dot{V}O_2$peak.

Following ∼36-48 h of recovery each participant returned to the laboratory to complete a pre-training exercise trial. Participants arrived at 16:30 h following a standardised meal and 4 h fast and rested in the supine position for 15 min prior to a pre-exercise blood sample and pre-exercise muscle biopsy. Immediately following, participants completed a concurrent exercise training protocol consisting of a ∼21 min squat protocol (outlined below), 15 mins passive rest and a ∼17 min repeated-sprint protocol (outlined below). The order of RT and RSA within the concurrent regime was chosen based on findings from previous work (Chapter 5). An immediately post-exercise venous blood sample was drawn at the cessation of exercise, and subsequent venous blood samples were drawn at 15 min, 60 min and 180 min post-exercise (Figure 7.1). Skeletal muscle biopsies were taken at 60 min and 180 min post-exercise (Figure 7.1).

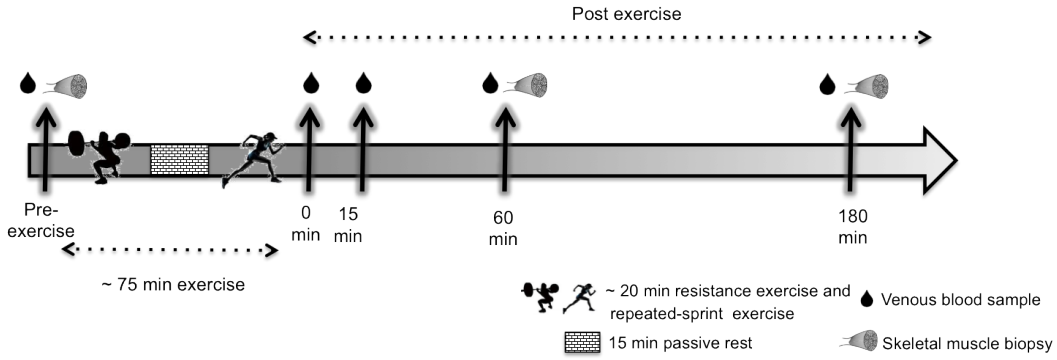


Figure 7.1: Overview of pre and post 4 weeks concurrent RT and RSA training acute experimental protocol.

Two days (48 h) post the pre-training exercise trial participants began their 4 weeks of training. The Same-Day training group (SDT) completed 3 concurrent resistance and repeated-sprint training (15 mins apart/same session) sessions a week for 4 weeks (total of 12 training sessions, Figure 7.2). The Alternative-Day training group (ADT) performed 6 sessions per week of alternating single mode resistance exercise or repeated-sprint exercise for the 4 week period (same total volume of training for each group, Figure 7.2).

Thirty six-48 h following the last training session participants came back to the laboratory for anthropometric measures and to perform the 1-RM strength testing of the back squat exercise, 30 m maximal sprint test and GXT. Two days (48 h) following post training performance measures participants came back in to the laboratory for the final visit at 16:30 h for the post training acute exercise trial (protocol was identical to the pre-training exercise trial).

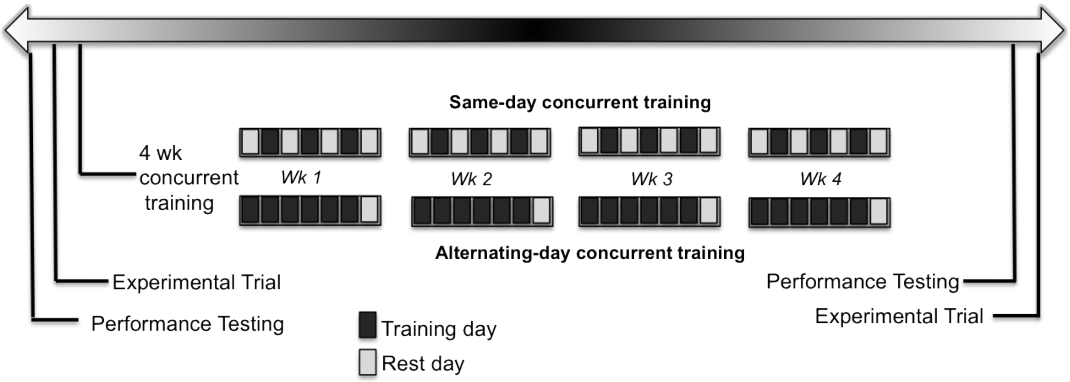


Figure 7.2: Overview of experimental procedures and 4 week concurrent RT and RSA training structure.

Menstrual and contraceptive control

During the participants initial visit to the laboratory it was confirmed that they were currently taking a monophasic combined oral contraceptive (OC) pill (Levonorgestrel 0.15mg and Ethinyloestradiol 0.03mg) and were also required to have had a regular menstrual cycle prior to OC use. Participants were instructed to remain on the active hormone tablets of their OC for the full duration of the study.

1-RM testing

Participants were required to undergo a 1-RM test for the back squat exercise as outlined by Vingren et al. [284] as a measure of maximal leg strength and for the determination of the load to be lifted during the experimental RT trial (80 % 1-RM). Participants completed a warm up comprised of squats for 8-10 repetitions at 50 % of their estimated 1-RM followed by another set of 2-5 repetitions at 85 % of estimated 1-RM. Following the warm up 4 to 5 1-repetition trials were used to determine the 1-RM. Lifts were considered successful once the top of the quadriceps had descended to be parallel with the floor. The reliability of this test in our laboratory is high; ICC = 0.97 and CV = 2.5 %.

Graded exercise test

$\dot{V}O_2$peak was determined using an incremental running graded exercise test on a motorised treadmill (Saturn 4.0, h/p/ Cosmos Sports and Medical GmbH, Nussdorf-Traunstein, Germany) at an incline of 1 %. Stages were 2.5 min long beginning at a speed of 8 km.h^{-1}. Initially the speed of the treadmill was increased by 2 km·h^{-1} to 10 km·h^{-1} and then for all subsequent stages treadmill speed was increased by 1 km·h^{-1} until volitional exhaustion was achieved. Strong verbal encouragement was

provided to participants as they came to the end stage of the test to ensure a maximal effort. A calibrated metabolic gas analysis system (PARVO; Parvo Trueone, Sandy, Utah)) was used to measure pulmonary gas exchange by determining ventilation and O_2 and CO_2 concentrations. Achievement of $\dot{V}O_2$peak was confirmed with a respiratory exchange ratio above 1.10, and HR within 10 beats of predicted maximal HR.

Concurrent repeated-sprint and resistance exercise protocol

Experimental squat protocol

Preceding the squat protocol participants were required to complete 1 set of 6 repetitions with the standard 20 kg Olympic bar followed by 1 set of 6 repetitions at 50 % of their experimental load, followed by another set of 6 repetitions at ∼70 % of their experimental load.

The resistance protocol consisted of 6 sets of 6 repetitions with 80 % of 1-RM for the back squat exercise with 3 min of passive rest between sets.

Experimental repeated-sprint protocol

Ahead of commencing the RSA sprint protocol, participants completed a warm-up of 6 sub-maximal sprint shuttle efforts followed by 6 progressive sprints of subjective intensities of ∼60,∼70 and ∼80 % of maximum effort. The RSA protocol was completed over ∼17 mins and was divided into 4 × 2 min blocks of maximal sprinting and active and passive rest. Each block comprised 6 all-out 20 m shuttle sprints (10 m there and back) departing every 20 s which were interspersed by ∼15 s of active (walking back to the start line) and passive rest (stationary standing). Sprint blocks were separated by 3 min passive rest and participants were able to drink water *ad libitum*. Electronic timing gates (Speed Light Sports timing system, SWIFT, NSW) were placed at the start/finish line to measure velocity (ms^{-1}) and time (s) for each sprint.

Exercise and dietary controls

Participants were instructed to avoid consuming alcohol and caffeine within the 24 h prior to both pre and post-training exercise trials and performance testing. To ensure standardisation of nutritional status between trials participants completed a 48 h food diary the day prior to and the day of the pre-training exercise trial and were asked to follow this as similarly as possible for the post-training exercise trial. All participants were provided with a standardised meal (∼ 51 KJ energy per

kg of body mass; ∼78 % CHO, ∼8 % Fat, ∼14 % Protein) that was to be eaten by 13:30 h on the day of the trial followed by a 4 h fast prior to commencing the trial. Participants were also supplied with 3 nutritionally identical breakfast options (∼27 KJ energy per kg of body mass; ∼85 %CHO, ∼5 % Fat, ∼10 % Protein) to be eaten on the morning of the trial. During the training period participants were instructed to avoid consuming sports drink during training (water was permitted) and were asked to refrain from eating within 90 minutes prior to and post training to reduce dietary and nutritional effects on training adaptation. All experimental trials took place between 16:30 and 22:00 h (exercise between 17:00 and 18:30 h). Therefore participants were required to complete all training sessions between the hours of 17:00 and 20:00 h. All performance testing was conducted at the same time of day for each participant. Participants were instructed that recovery from exercise should consist of passive rest only and that no other recovery modalities were to be used (i.e. the use of massage or anti-inflammatory products).

Participants were asked to refrain from any physical exercise within the 24 h prior to exercise trials and were asked to complete an exercise diary, recording any and all exercise performed 2 days prior to the pre-training exercise trial.

Blood collection and analysis

Blood collection

An indwelling venous cannula was inserted into an antecubital forearm vein with participants in the supine position. Pre-exercise venous blood was drawn via syringe into an 8 mL SST II (BD, Auckland, NZ) and 10 mL K_2EDTA (BD, Auckland, NZ) containing vacutainers. SST II vacutainers were allowed to clot at room temperature for 30 min and then centrifuged at 2500 rpm for 15 min, while K_2EDTA vacutainers were centrifuged immediately after withdrawal. After centrifugation serum and plasma was pipetted in to 300 ul aliquots before being stored at -80°C until required for analysis. Subsequent venous blood samples were drawn with participants in the supine position immediately post-exercise, 15 min, 60 min and 180 min in to recovery. Figure 7.3 summarises the specific points at which blood was drawn, and what blood draws were used for which analysis.

Serum hormone analysis

Insulin like growth factor (IGF-1), growth hormone (GH) and cortisol concentrations were quantified employing a direct solid-phase enzyme-linked immunosorbent assay (ELISA). ELISA kits (DRG Diagnostics, Germany) were used as per the included instruction booklet. All samples from the same participant were analysed on the

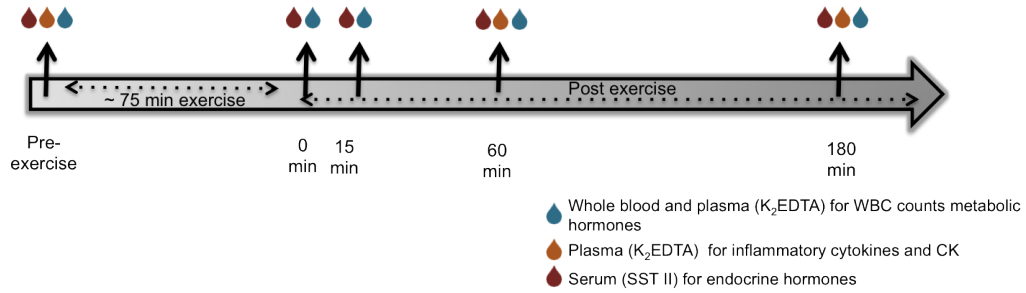


Figure 7.3: Time course of blood draws for analysis of endocrine and metabolic hormones, inflammatory cytokines and creatine kinase.

same ELISA plate. The intra-assay coefficients of variation (CV) were all under 5 % for IGF-1, GH and cortisol.

Plasma hormone analysis

Plasma concentrations of C-peptide, insulin, and glucagon were analysed with a Milliplex human metabolic hormone magnetic bead panel kit (Cat HMHMAG-34 K) according to the manufacturer's instructions except samples were analysed in singleton. Intra-assay CV's were under 5 %.

Glucose and creatine kinase

The plasma concentrations of glucose and creatine kinase (CK) were enzymatically measured using a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation).

Total and differential leukocyte counts

Leukocyte numbers were measured in K_2EDTA whole blood and analysed with a haematology analyser (Ac.T^{TM} 5 diff analyser, Beckman Coulter, NZ). Leukocytes included all counts of white blood cells including neutrophils, lymphocytes, and monocytes. All counts were adjusted for changes in plasma volume prior to analysis [308].

Skeletal muscle biopsy

The skeletal muscle biopsy involved the administering of a local anaesthetic (1 % Xylocaine) to the outside part of the upper thigh. It was ensured the local anaesthetic was administered superficially to avoid disruption of skeletal muscle signalling pathways. This was followed by a small incision made through the skin (∼1 cm in length). A 5 mm Bergstrum biopsy needle was then inserted through the incision

and into the vastus lateralis muscle in order to extract a small piece of muscle tissue (∼80-100 mg). Finally, the incision was closed with steri-strips. The second skeletal muscle biopsy was located on the same leg 2 cm proximal to the previous biopsy. The third skeletal muscle biopsy was performed on the opposite leg so that the resting and 60 min post-exercise skeletal muscle biopsies were performed on the same leg, with the 180 min skeletal muscle biopsy performed on the opposite leg. Each participant was randomly selected as to which leg (dominant, non-dominant) was biopsied first, with the opposite leg biopsied first for the post-exercise trial. All skeletal muscle biopsies were taken with participants in the supine position. Skeletal muscle samples were immediately removed from the biopsy needle, blotted (to remove blood), placed in liquid nitrogen and stored at -80°C until analysis.

Western blotting

Skeletal muscle biopsy samples were taken from storage in a -80°C freezer and placed in to a mortar and pestle embedded in dry ice and broken in to 2 pieces, approximately 30 mg of muscle was then placed in to a separate eppendorf on dry-ice for protein analysis. Seven 2.8 mm beads (Beads 19-646, Zirconium Oxide Bead Material, RNase/DNase free, Serial Number CB12F29) were then added to the 30 mg skeletal muscle biopsy samples. Ice cold RIPA lysis buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mmol/L EDTA supplemented with a cocktail of protease and phosphatase inhibitors including 1 mmol/L PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 mmol/L Na3VO4, and 1 mmol/L NaF) was added to 30 mg skeletal muscle biopsy samples at a ratio of 15 μL per mg of muscle and homogenised from frozen for 2 $\times$ 30 s at 5.65 s (Omini Bead Ruptor, Omni International, Inc.). Samples were then mixed in a freezer room for 60 mins (Programmable rotator-mixer PTR-35) before homogenates were pipetted in to new eppendorfs. Samples were then spun down at 13,000 g at 4°C for 20 min. The homogenate was separated from the pellet in to separate eppendorfs and frozen overnight at -80°C. Homogenates were thawed on ice and extracted proteins were quantified in triplicate using a BCA protein assay kit (Pierce, Auckland, New Zealand). All samples were then diluted with lysis buffer to a total volume of 400 μL and a concentration of 3 μg/μL muscle. Samples were then divided in to 2 aliquots of 200 μL, 1 aliquot was placed in -80°C for storage while the remaining 200 μL aliquot was prepared for boiling. 66.5 mL of Laemmli buffer and dithiothreitol (DDT) was added to each aliquot and then protein was boiled for 7 mins at 99.5°C and vortexed. 12.5 μL aliquots were pipetted in to 10 sets of eppendorfs and stored in a -20°C freezer. Each set of aliquots was then used once for western blotting. Aliquots were thawed once only, reboiled at 99.5°C, vortexed and centrifuged for 15 s. Protein homogenates (20 μg) were then separated by 6-15 %

SDS-Page and transferred (Transfer system, Bio-Rad Trans-Blot Turbo at 2.5 A, 25 v, 10 min × 2) to polyvinylidine fluoride membranes (Trans-Blot Turbo Transfer Pack, Midi Format, 0.2 um PVDF) blocked with 5 % bovine serum albumin (BSA), washed with 0.1 % Tris Base, sodium chloride, Tween 20 (TBST) for 3 × 10 min and incubated with primary antibody (rabbit or mouse) [p-Akt (Ser473), Akt, p-p70S6K (Thr389), p-GSK-3(Ser21/9), p-PRAS40(Thr246), p-STAT3(Ty705), 4E-BP1(Thr37/46), GAPDH; Cell signalling Technology (Danvers, MA)] at a 1:1000 dilution at 4°C overnight. Following overnight incubation membranes were washed in TBST for 3 × 10 min and incubated for 1 h at room temperature with a HRP-conjugated secondary antibody (anti-rabbit or anti-mouse) at a 1:2000-5000 dilution before being washed again in TBST for 3 × 10 min. Proteins were then detected via chemiluminescence and quantified by densitometry. Loading normalised western blot data was calculated as a fold change against participants respective baseline sample which was always run on contiguous lanes on the same gel.

Multiplex analysis for plasma and muscle cytokine concentration

Plasma and skeletal muscle protein expression of cytokines were analysed using a high sensitivity human cytokine kit (MILLIPLEX MAG, HSCYTMAG-60SK). Skeletal muscle samples were prepared as per western blotting and a validation of protein concentration versus kit sensitivity was carried out to determine the optimal protein concentration for detection prior to beginning analysis. All muscle samples were subsequently diluted to a concentration of 5.3 μg/μL. Plasma and skeletal muscle cytokines were analysed according to the manufacturer's instructions except samples were analysed in singleton. A multiplex assay was used for the simultaneous quantification of the following inflammatory cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon gamma (IFN-γ), IL-1β, Il-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13 and TNF-α. Samples were measured using Luminex xMAP technology (Luminex Corporation) and intra-assay CV's were all under 6 %.

Statistical analysis

For the analysis of performance variables, an ANCOVA was used to determine differences between groups from pre to post training, using the pre-training measure as a covariate to adjust for baseline differences between groups. Where no statistical differences were observable between the 2 training groups pre and post measures, groups were pooled and paired t tests were performed on the data (n= 12) to compare changes between pre and post training measures. All other data were analysed via linear mixed model with training status (pre or post training), training group and

time as fixed factors, and participants as a random factor. The trapezoid method was used to calculate the area under the curve (AUC) inclusive of the 75 min exercise protocol and 180 min of recovery for systemic cytokines and hormones. Where no differences were found between training groups, differences in AUC between pre and post-training systemic responses were analysed by paired t test ($n = 12$). For positively skewed distributed variables, log-transformation was performed prior to statistical analysis. Results are presented as mean ± SD. For all analyses differences were considered significant at a level of $p < 0.05$.

Results

Performance

There were no significant differences in performance changes between SDT and ADT training groups. 30 m sprint time was significantly reduced by 3.3 ± 2.3 %, ($p < 0.01$), 1-RM relative to body weight increased by 24.8 ± 5.9 % ($p < 0.01$) while $\dot{V}O_2$peak was unchanged -4.3 % ± 5.3, ($p = 0.217$), with 4 weeks of concurrent training irrespective of training group (Table 7.2).

Table 7.2: Changes in performance pre and post 4 weeks of concurrent RT and RSA training in female team-sport athletes.

Test	Group	Pre	Post	% Change
30 m Sprint (s)	Pooled	4.81 (0.2)	4.67 (0.2)*	-3.3 (2.3)
1-RM (rel. B/W)	Pooled	1.01 (0.14)	1.26 (0.16)*	24.8 (6.1)
$\dot{V}O_2$peak (mL·kg^{-1}·min^{-1})	Pooled	44.4 (4.0)	42.5 (4.5)	-4.3 (5.3)

Data presented as means (SD). *Significantly different from pre-training, $p < 0.05$.

Inflammatory cytokines

Circulating

Plasma cytokines are summarised in Tables 7.3, 7.4 and 7.5 and Figures 7.4 and 7.5. There was no effect for training status (pre-post-training) on the plasma cytokine response to an acute bout of RT:RSA concurrent exercise for any of the measured cytokines. There was an interaction effect (training group × time) for TNF-α at 180 min post-exercise irrespective of training status (pre to post-training) between the ADT and SDT training groups ($p = 0.02$) (Table 7.5). There were no training group (SDT or ADT) effects for any of the other plasma cytokines. However, there

were main effects for time, at 60 min recovery for the immuno-modulator IL-6, anti-inflammatory cytokines IL-10, IL-5, IL-7, GM-CSF and pro-inflammatory cytokines IL-2, IL-12, TNF-α, IFN-γ and IL-8, which were all significantly raised above pre-exercise levels (all $p < 0.02$) and remained elevated at 180 min in to recovery. IL-4 and IL-1β were also elevated above pre-exercise values at 180 min in to recovery (main effect for time, both $p < 0.05$). No differences in AUC response pre to post-training were observed for any of the plasma cytokines ($p > 0.05$).

Table 7.3: Anti-inflammatory systemic cytokine responses to acute concurrent RT and RSA exercise pre and post 4 weeks of concurrent RT and RSA training in female team-sport athletes.

Anti-inflammatory cytokines (pg/mL)	**Training**	**pre-exercise**	**60 min post-ex**	**180 min post-ex**
IL-6	Pre	1.08 (1.54)	2.78 (1.24)*	5.56 (1.65)*
	Post	1.09 (1.60)	3.05 (2.15)*	4.93 (1.96)*
IL-4	Pre	21.21 (2.00)	22.33 (1.60)	31.67 (1.75)*
	Post	18.35 (1.97)	25.93 (1.51)	32.97 (1.72)*
IL-10	Pre	12.59 (1.56)	17.87 (1.27)*	19.80 (1.53)*
	Post	10.44 (1.85)	20.95 (1.50)*	21.03 (1.97)*
IL-5	Pre	0.78 (1.85)	0.90 (1.52)*	1.22 (1.87)*
	Post	0.57 (1.98)	1.11 (1.54)*	1.22 (1.97)*
IL-7	Pre	2.44 (1.86)	3.17 (1.67)*	3.89 (1.58)*
	Post	2.19 (1.92)	3.61 (1.37)*	3.64 (2.01)*
GM-CSF	Pre	5.64 (2.23)	6.23 (1.82)*	8.60 (1.89)*
	Post	4.31 (2.48)	7.24 (1.70)*	8.27 (2.21)*

As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. n= 12) for pre to post training are presented. Data presented as mean (SD). *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$.

Local

GM-CSF, IFN-y, IL-1β, IL-2 and IL-4 had large numbers of samples (>40 %) below the detectable range. There were no effects of concurrent RT and RSA exercise on the skeletal muscle cytokine expression of IL-13, IL-6, IL-10, IL-8, IL-7, IL-12 or IL-5 regardless of training group (SDT or ADT) or training status (pre-post-training). However, there was a main effect for time ($p < 0.05$) for TNF-α which was significantly increased at 180 min ($p = 0.016$) post-exercise when compared to pre-exercise (Figure 7.5). There was also a main effect for time for IL-8 protein

Table 7.4: Pro-inflammatory systemic cytokine responses to acute concurrent RT and RSA exercise pre and post 4 weeks of concurrent RT and RSA training in female team-sport athletes.

Pro-inflammatory cytokines (pg/mL)	Training	pre-exercise	60 min post-ex	180 min post-ex
IL-2	Pre	3.23 (2.21)	3.59 (1.68)⋆	5.27 (1.68)⋆
	Post	2.34 (2.33)	4.39 (1.77)⋆	5.01 (2.04)⋆
IL-1β	Pre	0.80 (2.31)	0.81 (1.87)	1.21 (1.81)⋆
	Post	0.58 (2.77)	0.99 (1.82)	1.15 (2.27)⋆
IL-12	Pre	5.97 (2.14)	7.78 (1.41)	10.47 (1.51)⋆
	Post	5.37 (2.57)	9.81 (1.33)	10.89 (1.74)⋆
IFN-y	Pre	9.46 (2.05)	11.40 (1.65)⋆	16.29 (1.94)⋆
	Post	8.42 (2.50)	13.98 (2.17)⋆	15.60 (2.68)⋆
IL-13	Pre	0.83 (3.66)	1.35 (3.37)⋆	1.87 (4.16)⋆
	Post	0.70 (5.08)	1.61 (4.03)⋆	2.85 (4.02)⋆
IL-8	Pre	2.31 (1.59)	3.19 (1.55)⋆	3.17 (1.67)⋆
	Post	1.90 (2.03)	2.91 (1.82)⋆	3.27 (1.97)⋆

As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. n= 12) for pre to post-training are presented. Data presented as mean (SD). *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$.

Table 7.5: Systemic TNF-α responses to acute concurrent RT and RSA exercise pre and post 4 weeks of concurrent RT and RSA training between the SDT and ADT training groups.

Pro-inflammatory cytokine (pg/mL)	Training Group	Training	pre-exercise	60 min post-ex	180 min post-ex
TNF-α	SDT$^{\#}$	Pre	4.75 (1.38)	5.70 (1.18)⋆	5.53 (1.15)⋆†
	SDT $^{\#}$	Post	4.09 (1.31)	5.43 (1.15)⋆	5.93 (1.76)⋆†
	ADT	Pre	5.99 (1.34)	6.79 (0.60)⋆	6.45 (1.02)⋆
	ADT	Post	6.45 (1.83)	7.25 (1.46)⋆	7.09 (2.16)⋆

Data presented as mean (SD). *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$. $^{\#}$Significant training group effect; $p < 0.05$. †Change from pre-exercise significantly different to ADT at 180 min post-exercise; $p < 0.05$.

concentration which was significantly increased at 60 min post-exercise ($p = 0.016$) and 180 min post-exercise ($p = 0.0013$) when compared to pre-exercise (Figure 7.5).

Hormones

Results show that there were no effects for training group (SDT or ADT) for any of the hormones. However, there was a main effect for training status (pre-post-training) for cortisol, with the cortisol response being lower post the 4 weeks of training period compared with pre-training ($p < 0.001$), irrespective of training

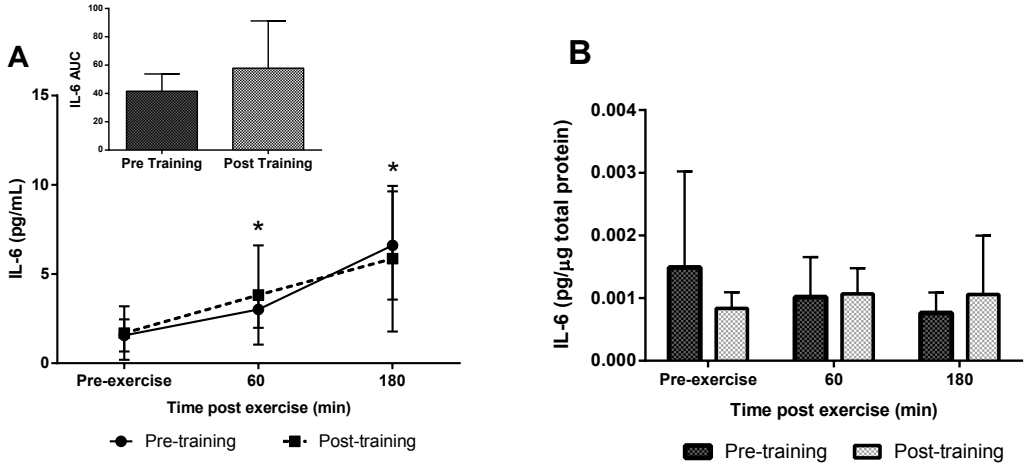


Figure 7.4: (A) Systemic versus (B) local (skeletal muscle) IL-6 responses to acute concurrent RT:RSA exercise pre and post 4 weeks of training. As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. $n = 12$) for pre to post-training are presented. Data presented as mean ± SD. *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$.

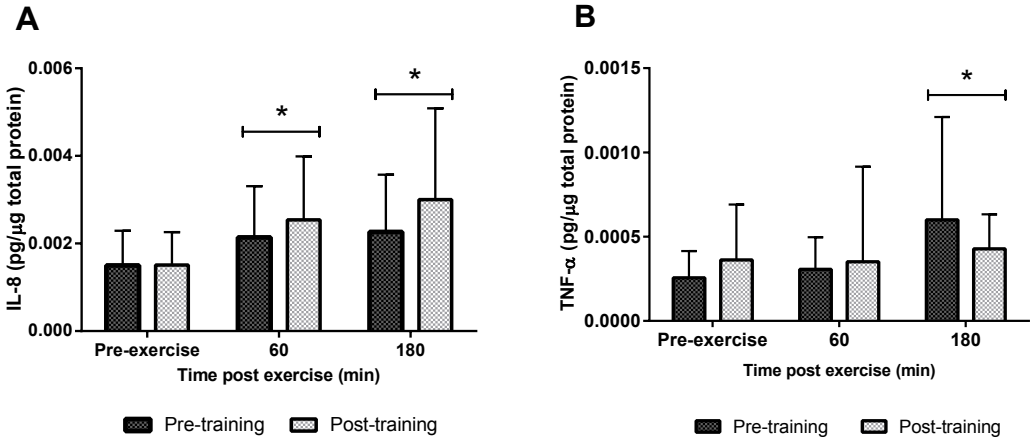


Figure 7.5: Local (skeletal muscle) (A) IL-8 and (B) TNF-α responses to acute concurrent RT and RSA exercise pre and post 4 weeks of training. As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. $n = 12$) for pre to post-training are presented. Data presented as mean ± SD. *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$.

group. AUC for cortisol was also lower following 4 weeks of training (pre-training: 1814.51 ± 687.50 vs. post-training: 1374.21 ± 570.04 ng·min·mL^{-1}, $p = 0.019$). There were also main effects for time for cortisol concentration which was increased above pre-exercise levels immediately post-exercise (pre-ex: 159.05 ± 50.40 vs. 0 min post: 216.60 ± 97.57 ng/mL, $p = 0.011$) reaching peak levels by 15 min post-exercise (256.05 ± 101.48 ng/mL, $p = 0.00$) and remained elevated at 60 min post-exercise (210.27 ± 94.75 ng/mL, $p = 0.033$) before dropping below pre-exercise levels by 180

min post-exercise (122.96 ± 67.05 ng/mL, $p = 0.00$; Figure 7.6).

There were no effects for training status (pre-post-training) but there were main effects for time ($p < 0.05$) for GH concentration which peaked immediately post-exercise (pre-ex: 5.19 ± 5.11 vs. 0 min post-ex: 20.08 ± 8.62 ng/mL, $p = 0.00$) and remained elevated at 15 min post (13.55 ± 6.32 ng/mL, $p = 0.00$) before dropping towards but still above pre-exercise values by 60 min post-exercise(6.01 ± 3.88 ng/mL, $p = 0.02$) and dropping below pre-exercise levels by 180 min post-exercise (2.01 ± 2.77 ng/mL, $p = 0.001$; Figure 7.6). There were no effects for training group (SDT or ADT), training status (pre-post-training) or time for IGF-1 concentrations ($p > 0.05$).

There was no effect for training status (pre-post-training) for glucagon. Main effects for time show glucagon increased above pre-exercise levels immediately post-exercise (pre-ex: 44.04 ± 20.34 0 min vs. post-ex: 70.85 ± 35.57 pg/mL, $p = 0.000$) and had returned towards pre-exercise levels by 15 min post-exercise (54.40 ± 26.64 pg/mL; Figure 7.7) where it remained stable.

There were no effects for training status (pre-post-training) for insulin or C-peptide. There was a main effect for time for insulin which was significantly raised above pre-exercise levels immediately post-exercise (pre-ex: 306.09 ± 313.37 vs. 0 min post-ex: 466.91 ± 413.54 ng/mL, $p = 0.012$). There was also a main effect for time for C-peptide which was significantly elevated above pre-exercise levels at 15 min recovery (pre-ex: 5848.82 ± 3588.64 vs. 15 min post-ex: 6515.89 ± 3921.54 ng/mL, $p = 0.026$). Both insulin (162.26 ± 136.25 ng/mL, $p < 0.001$; Figure 7.7) and C-peptide (4117.00 ± 2769.18 ng/mL, $p = 0.002$; Figure 7.7) dropped below pre-exercise levels by 60 min in to recovery.

Glucose

There were no effects for training group (SDT or ADT) on glucose response. There was a main effect for training status (pre-post-training) for glucose response which was lower post the 4 week training period compared with pre-training ($p = 0.01$). AUC for glucose was also lower following training (pre-training: 48.25 ± 7.70 vs. post-training 34.99 ± 5.55 mmol·min·L^{-1}, $p < 0.001$). There were also main effects for time, with glucose being elevated above pre-exercise levels immediately post-exercise (pre-ex: 4.79 ± 0.43 vs. 0 min post ex: 6.43 ± 1.29 mmol/L, $p = 0.002$) and remaining elevated at 15 min in to recovery (5.41 ± 1.43 mmol/L, $p < 0.001$) before returning to pre-exercise levels by 60 min in to recovery (4.50 ± 0.62 mmol/L, $p = 0.067$)).

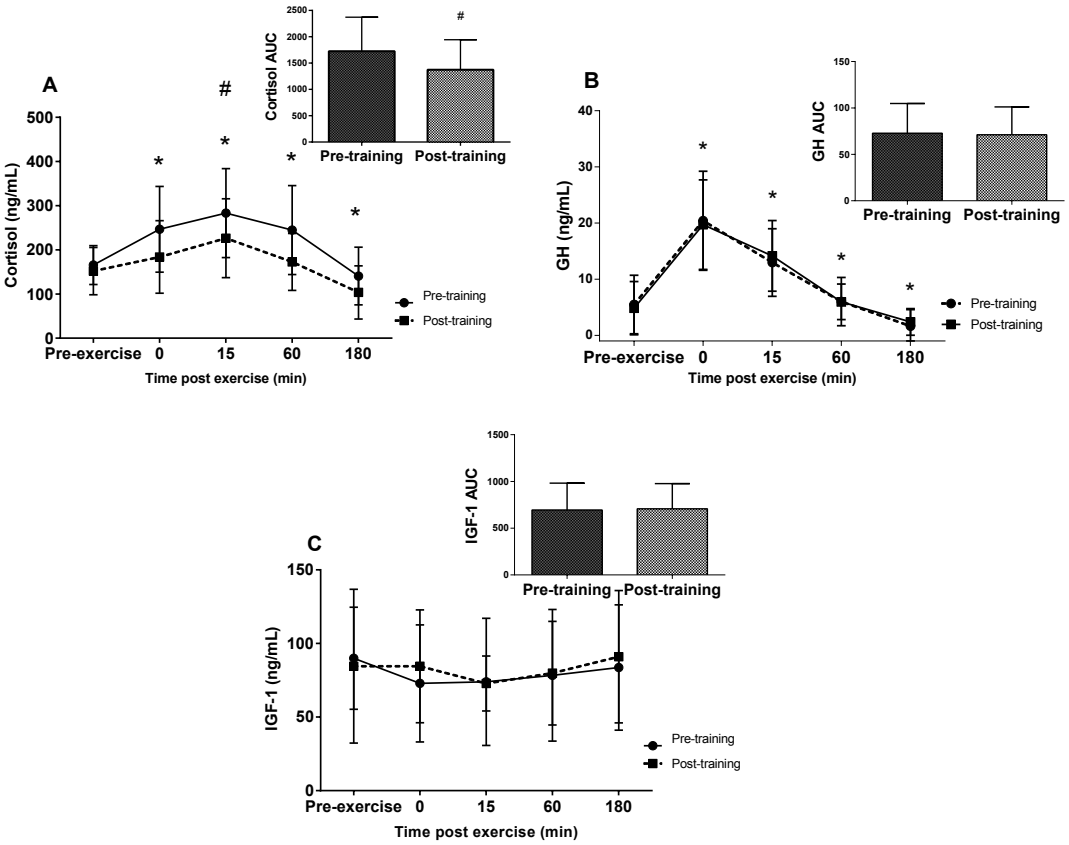


Figure 7.6: Serum (A) cortisol, (B) GH and (C) IGF-1 hormone responses to acute concurrent RT and RSA exercise pre and post 4 weeks of training in female team-sport athletes. As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. $n = 12$) for pre to post-training are presented. Data presented at mean ± SD. *Significantly different from pre-exercise concentration, main effect for time; $p < 0.05$. #Significantly different from pre-training, $p < 0.05$.

White blood cell mobilisation

White blood cell data are summarised in Table 7.6. There were no effects for training group (SDT or ADT) on circulating leukocyte, lymphocyte, neutrophil or monocyte counts. Total leukocyte count increased significantly from pre-exercise immediately following the exercise protocol and remained elevated up to 180 min post-exercise ($p < 0.001$) irrespective of training status. A significant training (pre-post-training) effect was observed in neutrophil count which was higher post-training ($p = 0002$). There were also main effects for time for neutrophil counts which were significantly increased immediately post-exercise ($p < 0.001$) and remained elevated up to 180 min post-exercise ($p < 0.008$) irrespective of training status. Main effects of time were also seen for circulating lymphocyte and monocyte counts which were significantly raised above pre-exercise values immediately post-exercise ($p < 0.001$) but had returned to pre-exercise levels by 15 min in to recovery.

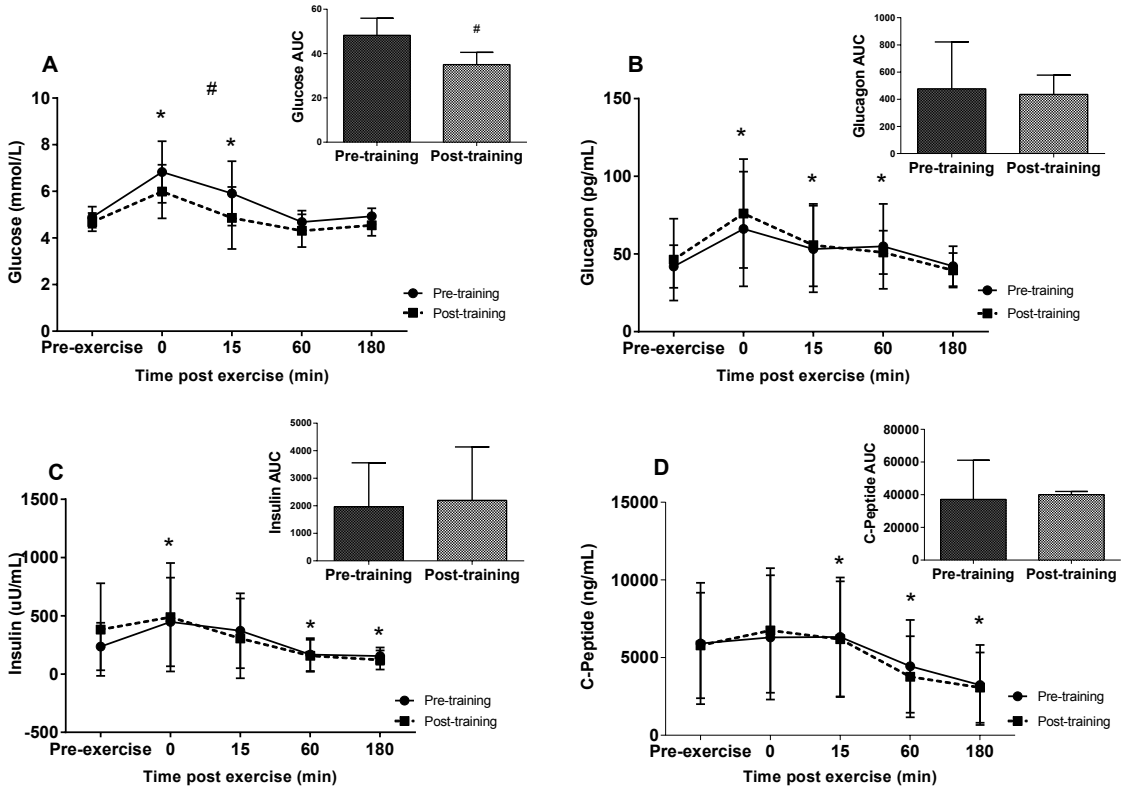


Figure 7.7: Plasma (A) glucose, (B) glucose, (C) insulin and (D) C-peptide responses to acute concurrent RT and RSA exercise pre and post 4 weeks of training in female team-sport athletes. As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. $n = 12$) for pre to post-training are presented. Data presented as mean ± SD. *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$. #Significantly different from pre-training, $p < 0.05$.

Table 7.6: Total leukocyte, lymphocyte, neutrophil and monocyte cell counts in female team-sport athletes following concurrent RT and RSA exercise both pre and post 4 weeks of training in female team-sport athletes.

White Blood Cells cytokines (10^9 cells/L)	**Training**	**Pre exercise**	**0 min post-ex**	**15 min post-ex**	**60 min post-ex**	**180 min post-ex**
Total leukocytes	Pre	6.7 (1.2)	11.3 (1.0)*	7.9 (1.0)*	10.0 (1.7)*	10.7 (1.2) *
	Post	7.1 (1.0)	12.0 (1.2)*	8.8 (1.8)*	9.5 (1.6)*	10.4 (1.4)*
Lymphocytes	Pre	2.5 (0.6)	4.6 (1.5) *	2.6 (0.5)	2.2 (0.5)	2.7 (0.7)
	Post	2.6 (0.5)	4.8 (1.3)*	3.3 (0.7)	2.3 (0.6)	2.6 (0.7)
Neutrophils	Pre	3.5 (0.9)	5.1 (1.1)*	4.6 (1.1)*	4.5 (0.9)*	6.5 (2.6)*
	Post$^\#$	4.4 (1.6)	6.6 (3.0)*	6.9 (2.0)*	5.7 (3.1)*	6.9 (2.7)*
Monocytes	Pre	0.5 (0.1)	0.9 (0.1)*	0.5 (0.1)	0.5 (0.1)	0.5 (0.2)
	Post	0.6 (0.1)	1.0 (0.2)*	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)

As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. n= 12) for pre to post-training are presented. Data presented as mean (SD). $^\#$Significant training (pre to post) effect, $p < 0.05$. *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$.

Creatine kinase

Plasma CK levels were unaffected by either training group (SDT or ADT) or training status (pre-post-training). However, there was a main effect for time ($p < 0.05$) and exercise did cause elevations from pre-exercise CK levels at both 60 min (pre-ex: 158.57± 132.29 vs. 60 min post-ex: 196.26 ± 141.71 U/L, $p = 0.04$) and 180 min post-exercise (239.96 ± 157.69 U/L, $p < 0.001$; Figure 7.8).

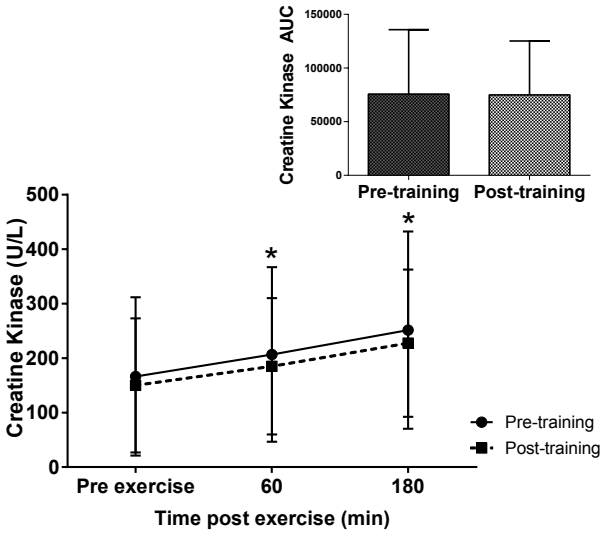


Figure 7.8: Alterations in creatine kinase to concurrent RT and RSA exercise pre and post 4 weeks of training in female team-sport athletes. As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. $n = 12$) for pre to post-training are presented. Data presented as mean ± SD. *Significantly different from pre-exercise concentration; main effect for time, $p < 0.05$.

Signalling responses

The measured skeletal muscle signalling proteins were largely unaffected by exercise. There were no significant effects for training group (SDT or ADT) or training status (pre-post-training) for any of the measured proteins (Figure 7.9). There was a main effect for time for 4E-BP1 activity which was significantly suppressed at both 60 min and 180 min post-exercise when compared with pre-exercise ($\sim$ 0.7 fold, $p < 0.009$; Figure 7.9), while a significant main effect for time showed that p70S6K activity was up-regulated at 60 min post-exercise ($\sim$1.4 fold, $p = 0.048$; Figure 7.9) from pre-exercise. All other molecular proteins showed no significant change in phosphorylation status from pre-exercise levels at 60 or 180 min post-exercise.

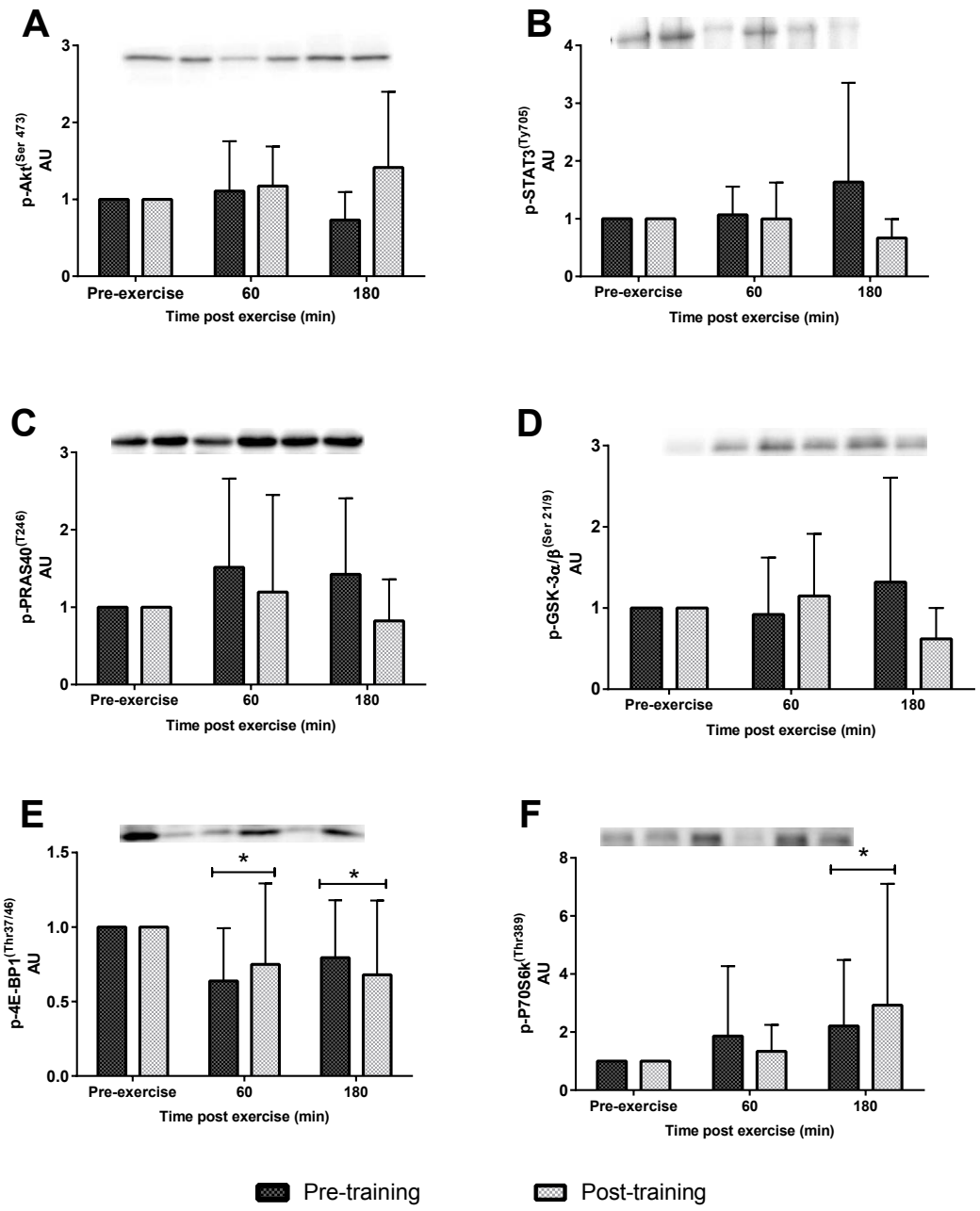


Figure 7.9: Skeletal muscle signalling responses to acute concurrent RT:RSA exercise pre and post 4 weeks of training in female team-sport athletes. (A) p-Akt (Ser473), (B) p-STAT3(Ty705), (C) p-PRAS40(Thr246), (D) p-GSK-3(Ser21/9), (E) 4E-BP1(Thr37/46), (F) p-p70S6K (Thr389). As there were no significant differences evident between the ADT and SDT training groups, pooled data (i.e. $n = 12$) for pre to post-training are presented. Data presented as fold change from pre-exercise values, mean ± SD. p70S6K and 4E-BP1 $n = 11$, unable to detect band for one individual. * Significantly different from pre-exercise levels; main effect of time, $p < 0.05$.

Discussion

An important finding of the current study was that the configuration of concurrent RT and RSA training did not effect acute post-exercise physiological or performance responses to concurrent RT and RSA exercise following 4 weeks of concurrent train-

ing. It was anticipated that the greater acute training stimulus performed during training in the SDT group and the familiarity with performing both modes of exercise within the same session may increase the adaptive stimulus for performance and reduce the physiological stress during the post-training trial to a greater extent when compared with the ADT training group

As well, training status (pre-post-training) only altered glucose and cortisol responses to acute exercise and therefore 4 weeks of concurrent RT and RSA training had little effect on overall physiological responses in trained female team-sport athletes. The results of the present study did however demonstrate that an acute bout of concurrent RT and RSA exercise in well-trained female team-sport athletes rapidly and transiently increased markers of metabolism and endocrine hormones in the immediate recovery period. Concurrent exercise also caused an elevation of both pro- and anti-inflammatory cytokines at 60 min post-exercise in the circulation which continued to rise by 180 min post-exercise. As well, skeletal muscle protein concentration of IL-8 (60 min and 180 min) and TNF-α (180 min) were elevated by acute concurrent exercise irrespective of training status. Interestingly, no changes were observed in skeletal muscle protein concentration of IL-6 following acute concurrent RT and RSA training irrespective of training status. Of the molecular signalling proteins measured, only 4E-BP1 (modestly depressed) and p70S6K (modestly increased) protein were altered by concurrent exercise.

One of the aims of the current study was to investigate changes in protein expression of pro- and anti -inflammatory cytokines within the exercised skeletal muscle to concurrent RT and RSA exercise prior to and following 4 weeks of SDT or ADT concurrent training, and whether they mimic changes seen in circulating cytokines. Prior to training acute post-exercise protein expression of inflammatory cytokines were not different between the SDT and ADT training groups and training structure (SDT or ADT) did not have any effect on the post 4 week training acute concurrent exercise response. Concurrent exercise increased the protein expression of the major pro-inflammatory cytokine TNF-α at 180 min post-exercise ($\sim$2 fold) and the chemokine IL-8 at 60 min and 180 min post-exercise ($\sim$2 fold). Previous literature has reported marked elevations in skeletal muscle mRNA of IL-6, IL-8, IL-15 and TNF-α, following acute resistance [201, 202, 215] and running exercise [202, 204]. In addition, a recent study by Della Gatta et al. [155] showed marked elevations in protein expression of IL-8 ($\sim$28 fold) and IL-6 ($\sim$4 fold) and undetectable levels of TNF-α 2 h post leg-extension exercise measured in muscle homogenates and analysed by bio-plex array similarly to the current study.

An interesting finding in the current study was the lack of change in IL-6 protein expression in skeletal muscle following acute concurrent RT and RSA exercise despite a $\sim$5 fold increase in plasma IL-6 concentration. IL-6 has been shown to be

produced by contracting skeletal muscle during exercise of varying modes, intensities and durations [37, 39, 136, 155, 156]. It is now widely accepted that the major source for the rise in circulating IL-6 during exercise is the contracting skeletal muscle itself [136]. However, others report that skeletal muscle release of IL-6 cannot alone explain the increase in plasma IL-6 during exercise [142]. It is hypothesised that IL-6 is released from the muscle into the circulation during exercise in response to lowering muscle glycogen stores, where it may act in an endocrine like manner initiating cross-talk with the liver in order to increase blood glucose [32]. In support of this theory, IL-6 mRNA and circulating levels are further enhanced when muscle glycogen stores are low [146, 279], and CHO ingestion has been shown to attenuate the increase in circulating IL-6 [37, 204].

In the current study significant increases in plasma IL-6 at 60 min post-exercise and a continued rise at 180 min post-exercise (∼5 fold) were observed, but no significant change in IL-6 protein expression in skeletal muscle at concomitant time points was reported. Following the same exercise protocol as the current study, plasma IL-6 was found to be significantly elevated above pre-exercise levels immediately post-exercise (Chapter 4). Previous investigations have shown increases in mRNA gene expression of IL-6 within 3 hours of exercise cessation [37, 182, 293], while Della Gatta et al. [155] found significant increases in IL-6 protein expression (∼4 fold) 2 h following 3 × 12 sets of maximal unilateral knee extension in untrained and fasted young and old men. Interestingly, in the current study lower cumulative (AUC) blood glucose concentration and cortisol concentrations were found following 4 weeks of concurrent training, suggestive of lower metabolic demand. Yet despite these changes there was no difference in the plasma IL-6 response to acute concurrent exercise post 4 weeks of training.

The participants in the current research were trained team-sport athletes who were provided with a high-CHO meal (∼78 % CHO) 4 h prior to exercise and were therefore not fasted and likely to have full glycogen stores at the onset of exercise. In contrast, the participants in the Della Gatta et al. [155] study arrived at the laboratory in a fasted state. It is possible that these differences in nutritional status may account for the lack of change in IL-6 protein expression in skeletal muscle in the current study compared to that seen by Della Gatta et al. [155]. Also, following the same exercise protocol as the Della Gatta et al. [155] study and with similar participants, Trenerry et al. [156] report between 5-17.5 fold increases in IL-6 skeletal muscle protein concentration following acute exercise at 3 h in to recovery. These results suggest that the timing of the muscle collection may be important, and/or that there appears to be variability in the magnitude of changes in IL-6 protein abundance within similar populations.

Muscle glycogen stores were not measured in the current study, but it is

possible that glycogen stores did not deplete to sufficient levels to be considered an energy crisis and therefore muscle-liver cross talk was not required. It may also be possible that the mechanism driving elevation of plasma IL-6 during and post-exercise differ, and that had IL-6 been measured in the skeletal muscle during exercise, alterations in IL-6 protein levels may have been seen. The current study has however, provided some evidence that the skeletal muscle production of IL-6 cannot account for the rise in plasma IL-6 during the recovery from exercise.

Skeletal muscle has been shown to have the capacity to express IL-8 in response to both concentric and eccentric contraction [39, 204] and this finding is supported by the current study. IL-8 plasma concentrations appear to be elevated predominantly during exercise with a strong eccentric component such as running [195,196], soccer [145] and concurrent RT:RSA exercise (Chapter 5) but not during predominantly concentric activity such as bicycle ergometer exercise [39]. The systemic IL-8 response is therefore most likely related to an inflammatory response where it acts as a chemokine on neutrophils [192]. Less is known of the role of IL-8 within skeletal muscle, but as only small amounts of IL-8, not significant enough to elevate plasma levels, have been released from the muscle, it is thought that skeletal muscle produced IL-8 exerts its effects locally [203]. IL-8 is part of the CXC family of chemokines, and it has been shown that CXC2 receptor mRNA and protein expression is up-regulated in human microvascular cells after concentric exercise [205]. Therefore it is thought that exercise stimulated IL-8 expression in skeletal muscle acts locally, attaching to the CXC2 receptor to stimulate angiogenesis in the endothelial microvasculature [203]. In the current study, the increase in IL-8 protein expression at 60 min following concurrent RT and RSA training was significantly lower (∼2 fold) than the exercise induced response to 3 × 12 reps of maximal leg extensor exercise at a constant speed of $60°s^{-1}$ (∼28 fold at 2 h post-exercise) seen by Della Gatta et al. [155]. Therefore it is difficult to tell if the exercise induced increase in the current study is physiologically significant.

TNF-α is a pro-inflammatory cytokine that has been shown to mediate muscle proteolysis, attenuating insulin-stimulated protein synthesis through alterations in translation initiation through a decrease in eIF4F complex assembly in myotubes [335, 336]. TNF-α has also been found to be inversely related to the rate of mixed muscle protein synthesis in older individuals [337].

The increase in TNF-α expression in skeletal muscle was small in the current study (∼2 fold). Although, taking this finding in combination with the low activation of the traditional muscle building pathways also seen in the current study, may be suggestive of a low anabolic environment within the immediate recovery period following concurrent RT and RSA exercise. More research in to the acute effects of TNF-α activity on acute skeletal muscle protein synthesis following exercise may

help to clarify the role of inflammatory cytokines in the architectural adaptation of skeletal muscle to exercise. It is likely that a local inflammatory response is required to promote healing and remodelling or tissue adaptation that otherwise may not occur. Understanding when inflammation may negatively affect muscle growth could provide important information for the exercise physiologist that might help determine post-exercise recovery techniques.

Differences in skeletal muscle concentrations of inflammatory cytokines between the present study and the Della Gatta et al. [155] and Trenerry et al. [156] studies may be due to the training background of the participants (trained team-sport athletes completing team-sport specific exercise vs. resistance untrained athletes completing maximal unfamiliar exercise), the targeted muscle groups (exercise distributed over lower-body musculature vs. exercise targeting only the quadriceps), type of contraction (concentric, eccentric), the timing of the post-exercise biopsy (180 min vs. 120 min post-exercise) or sex (female vs. male).

Attempts have been made to establish a role for the female sex hormone, estrogen, as a protective effector against exercise induced muscle damage [338]. However, findings for a protective role of estrogen in exercise induced muscle damage in humans are more equivocal than the findings in rodents, likely due to the difficulties in controlling extenuating biological factors, and thus the effect of this hormone remains unclear [339]. It has also been suggested that estrogen may not offer protective effects from actual muscle damage, but rather may regulate the post-injury repair processes by influencing the inflammatory response [338, 340]. The participants in the current research were taking a combined oral contraceptive and may therefore have had modestly increased estrogen levels which could possibly have had an effect on the cytokine response to concurrent RT and RSA exercise. However, as the cytokine response in both skeletal muscle and plasma in the current study were unchanged following 4 weeks of training, it seems possible that the cytokine presence in the immediate post-exercise recovery period is not necessarily contingent on muscle injury.

The present study also observed no effects for either SDT or ADT training or training status (pre-post 4 weeks of training) on the signalling response in our trained female population. Further, the only observed changes in phosphorylation status of signalling proteins was a lowered 4E-BP1 activity at 60 ($\sim$0.7 fold) and 180 min ($\sim$0.7) post-exercise and the modest increase in p70S6K activity 60 min ($\sim$1.3 fold) in to recovery when compared to pre-exercise activity. As 4E-BP1, an initiator of translation and p70S6k involved in the synthesis of new proteins, are downstream of both Akt and mTOR it is possible that in the current study the time points chosen for the muscle biopsy did not coincide with the peak signalling response to the concurrent exercise. The time course for muscle biopsies were based on informa-

tion from previous research that investigated the post-exercise signalling response to resistance [341, 342], endurance [74, 342], repeated-sprint [343] and concurrent training [72, 74].

The time course for cell-signalling following an acute concurrent regime consisting of resistance and repeated-sprint exercise is currently unknown [72] and to the author's knowledge this is the first study to investigate the signalling response to combined resistance and maximal ground running efforts. However, following similar repeated maximal sprint efforts to the current study (4 × 6 30 m sprint efforts), no changes were found in Akt phosphorylation and 4E-BP1 signalling was significantly depressed at 15 min in to recovery in male athletes, before returning to pre-exercise levels 2 h post-exercise, but was unchanged in females [344]. Similarly, following all out bicycle sprint exercise (4 × 30 s wingate) in healthy males, Akt tends to decrease and phosphorylation of 4E-BP1 and p70S6K phosphorylation remains unchanged immediately post or 3 h in to recovery [343]. In a concurrent repeated-sprint (10 × 6 s, 0.75 N·m torque·kg^{-1} (bicycle)) and resistance (8 × 5 leg extension, 80 % 1-RM) study in trained males, increases in ribosomal protein S6 (rpS6) 15 min following resistance exercise have been recorded but this was attenuated when resistance exercise was undertaken after repeated-sprints and exercise of either order diminished IGF-1 mRNA [72]. Therefore, it appears possible that maximal RSA exercise may not encourage the initiation of translation in the early post-exercise phase. Unfortunately, as there was no control group (isolated resistance and/or repeated-sprint group) within the current study it is not possible to discount an interference effect of concurrent lower-body resistance and maximal running repeated-sprint efforts on acute signalling within skeletal muscle. There does however appear to be no divergence in the acute signalling response to same-session concurrent RT and RSA exercise following SDT or ADT concurrent training.

This study represented a population of trained female athletes with many years of team-sport training experience. Even though they were not necessarily familiar with training both modes of exercise (RT and RSA) within the same session they did have some prior training experience in both forms of exercise. Therefore, it may be that in a trained population, the signalling response is more efficient and presents both an earlier and faster time course of activation and the timing of peak activation in the present study was unfortunately missed. However, without further investigation this remains speculative. Coffey et al. [345] has demonstrated that prior training attenuates the exercise specific molecular responses to single mode exercise in male strength-trained and endurance-trained athletes undertaking 1 h cycling (70 % $\dot{V}O_2$max) or 30 min (8 × 5 max reps of isokinetic leg extensions) resistance exercise. p70S6K and S6 phosphorylation were increased following resistance exercise only in endurance trained participants, while AMPK was increased only in strength trained participants following aerobic exercise. Therefore, training

status may determine the predominant signalling pathway to concurrent exercise and may provide an explanation as to why we observed a lack of stimulation in either the AMPK-PGC-1a, Akt-mTOR or JAK/STAT signalling pathways to concurrent training.

In response to contractile stimuli cytokines such as IL-6 are able to phosphorylate JAK and subsequently STAT3 signalling through the gp-130 receptor [346]. Once phosphorylated STAT3 is translocated to the nucleus where it can regulate target genes which are involved in the proliferation and differentiation of satellite cells [179, 347]. Leg extension exercise has been shown to be a stimulus for STAT3 and target gene activation at 2 and 3 h post-exercise in humans and is thought to be an important mechanism mediating repair, restructuring and satellite cell hypertrophy during the post-exercise recovery period as it appears to be unaltered by training [156]. However, the current study did not show evidence of alterations in skeletal muscle IL-6 or STAT3 phosphorylation following concurrent resistance and RSA exercise.

Additional investigations examining the time course and phosphorylation status of prominent signalling proteins to isolated and concurrent resistance and repeated-sprint exercise will improve the post-exercise analysis of the signalling mileu. Further, investigating the signalling response in populations differing by sex and training status are required to ensure the correct timing for the biopsy procedure for specific populations in future studies. While sedentary or untrained populations provide important information concerning initial adaptation to training the responses likely do not represent changes in trained athletes. To better understand the molecular mileu involved in adaptation specifically for functional performance adaptation in athletes, a greater number of studies using trained populations are required.

Interestingly, the acute systemic and skeletal muscle cytokine responses to concurrent exercise after 4 weeks of training were unaffected by exercise training group. Although both groups performed the same total volume of exercise, the rest periods between the two modes of exercise were vastly different (15 min vs. 24 h). Due to the different training structures of the two training groups in the present study it was expected that the SDT training group may have become more accustomed to the physical and metabolic demands of performing both modes of exercise within the same session. However, the circulating inflammatory cytokine response was unaffected by training irrespective of the structure in which training was carried out, while GH response was also unaffected by training and overall cortisol and glucose responses were lower post-training regardless of training group. As well, similar significant improvements in performance occurred independent of training structure.

The lack of major alterations in the inflammatory cytokine and signalling

responses after 4 weeks training suggest that these acute responses played only a nominal role in the adaptive process that led to the performance changes observed in the current study. Therefore performance changes may have been predominated by neuromuscular adaptation [85,86,91] and/or training task specific learning that may have led to mechanical or kinematic improvements [348,349]. It is however possible that the adaptive processes within the skeletal muscle continue beyond the duration of the current study and therefore the changes in acute cytokine and signalling responses may have remained intact. However, the lack of alteration in acute systemic and local cytokine responses in the current study are in agreement with previous studies with training durations of 2-12 weeks, who have also demonstrated significant performance improvements following leg extension or high-intensity exercise training without alterations in the inflammatory cytokine response [155, 156, 200]. It is suggested that the cytokine response to exercise may be an essential exercise induced process that maintains sensitivity to the acute stimulus following training. Also, the circulating inflammatory response was not mimicked by the skeletal muscle response within the time course specified in the current study. This may suggest that systemic inflammatory responses likely cannot easily provide information about local responses in skeletal muscle, though more research is this area is required to confirm this.

Conclusion

This is the first study to globally examine the hormonal, systemic and local inflammatory, and skeletal muscle signalling responses to concurrent training incorporating lower-body RT exercise followed by maximal repeated running sprints in a female team-sport population. A novel finding of the current study was that 4 weeks of concurrent RT and RSA training was unable to significantly alter the acute exercise induced physiological, hormonal or cytokine responses. Specifically, an array of circulating pro-(TNF-α, IFN-γ, IL-8, IL-2 and IL-12) and anti-inflammatory (IL-6, IL-10, IL-5, IL-7 and GM-CSF) cytokines were elevated during the 180 min of recovery from concurrent exercise, and skeletal muscle IL-8 (60 min and 180 min) and TNF-α (180 min) protein were elevated post-exercise. As well an immediate elevation of endocrine hormones (GH, cortisol, glucagon, insulin) and glucose were observed following exercise. p70S6K phosphorylation was moderately elevated at 60 min post-exercise regardless of training status, while 4E-BP1 phosphorylation was depressed in the 180 min following acute exercise. Only plasma glucose and serum cortisol were significantly altered by training. It would appear that the training period (4 weeks) was not long enough to elicit large scale physiological adaptation, and that perhaps had a longer training period been used an attenuation of some of these responses may have been seen. Also, it may be that some of these pro-

cesses are essential to the adaptive response to exercise and therefore are not varied by training. Interestingly, large and practical improvements in both strength and speed were reported for both training groups despite the lack of attenuation of the acute exercise induced stress/adaptive response which suggests that neuromuscular adaptations may have taken place in order to improve contractile efficiency and performance. An additional finding of the current study was that in contrast to previous reports, the increase in plasma IL-6 with exercise in the current study was not accompanied by increases in IL-6 protein within the skeletal muscle. While both cortisol and glucose responses were lower after 4 weeks of training, the systemic IL-6 response remained stable. SDT or ADT training did not differentially affect peripheral or local responses to acute same-session concurrent RT:RSA exercise. Therefore coaches and strength and conditioning practitioners can feel confident about prescribing either SDT or ADT concurrent training for comparable improvements in performance, physical preparedness and physiological tolerance of competitive match performance.

CHAPTER 8

The effect of circulating IL-6 on hepatic glucose production in an isolated liver model: proof of concept

Prelude

IL-6 is a myokine that is hypothesised to have a role in glucose metabolism during exercise, as discussed in Chapter 3. However, as described in Chapter 7, no significant changes were observed in skeletal muscle IL-6 protein concentration following concurrent RT and RSA exercise despite significant increases in plasma IL-6 up to 180 min in to post-exercise recovery. Alongside these findings, 4 weeks of training reduced both the glucose and cortisol response to acute concurrent RT and RSA exercise suggestive of lowered metabolic stress though interestingly, the plasma IL-6 response to acute exercise was unaltered by 4 weeks of training. While the measures in Chapter 7 may not have coincided with the peak timing for an IL-6 metabolic response, the role of IL-6 in glucose metabolism became less clear than before this thesis was started

It was decided that a novel method would be tested to determine the role of circulating IL-6 on HGO using a perfused isolated rat liver model. Consequently, the following aims were added to the thesis to be answered in Chapter 8.

- Test a novel method to determine the role of IL-6 on HGO (perfused isolated rat liver model).
- Test the effect of circulating IL-6 on HGO in combination with glucagon or adrenaline.
- Test whether an unknown 'contraction factor' works in syngergism with IL-6 during exercise to increase HGO.

Abstract

The mechanism underlying the increased rate of endogenous glucose production from the liver during exercise remains unclear. The cytokine interleukin-6 (IL-6) is known to be elevated during exercise and it is thought that circulating IL-6 either directly or via a 'contraction factor' stimulates the release of stored glucose in the liver. A novel method was used to test this theory by directly infusing rhIL-6 and possible 'contraction factors' through an isolated rat liver. It was shown that IL-6 did not directly increase hepatic glucose output (HGO) in the isolated rat liver. Moreso, IL-6 infused at the same time as glucagon caused a significant reduction in HGO, however, IL-6 infused with adrenaline caused no synergenic increase or decrease in HGO. To test if an unknown 'contraction factor' was needed along with IL-6 to increase HGO, human fasted and exercised plasma was perfused with or without rhIL-6 through in an isolated liver system. It was found that fasted and exercised plasma increased HGO, as expected, but infused with rhIL-6 significant and non-significant reductions in HGO were found. The results of the current study provide some evidence that circulating IL-6 does not increase HGO and may work as a negative regulator of HGO independent of an unknown 'contraction factor'.

Introduction

Exercise is a powerful stimulus for modulating rates of glucose disposal via increased skeletal muscle glucose uptake in order to sustain exercise intensity [350]. An increased rate of endogenous glucose production from the liver is essential to cope with the increased energy demand [351–355]. However, the mechanisms underlying the increased hepatic glucose production during exercise remain unclear.

Researchers have been trying to identify a skeletal muscle contraction induced factor that may mediate exercise induced physiological effects within other organs. Interleukin(IL)-6 has been identified as a myokine that is produced in and secreted by contracting skeletal muscle and has been suggested to play a role in exercise metabolism [33, 292]. It is reported that the exercise induced plasma IL-6 response is attenuated upon the ingestion of carbohydrate (CHO) [163] and IL-6 gene transcription within skeletal muscle is affected by intramuscular glycogen stores [37]. As well, direct exposure of IL-6 on skeletal muscle has been shown to cause an increase in the GLUT4 dependant-glucose uptake pathway [146,173,356–358]. Systemic IL-6 also shows an exercise duration and intensity dependent response to exercise [31–33]. It has therefore been hypothesised that IL-6 may contribute to the increase in HGO during exercise [33, 159, 359].

In support of this theory, Febbraio et al. [35] infused recombinant human (rh) IL-6 (∼10 pg/mL) during exercise in healthy young males and found the glucose rate of appearance and disappearance was higher during exercise with rhIL-6 infusion compared with exercise alone [35]. In contrast, Steensberg et al. [170] studied both (in the context of exercise induced responses) extreme (∼143 pg/mL) and supra-physiological (∼319 pg/mL) concentrations of rhIL-6 infusion in healthy young men in a resting state and found no increase in whole body glucose disposal or any increase in endogenous glucose production [170]. These contrasting human studies suggest there may be a secondary exercise factor that works as a synergist with IL-6 to increase endogenous glucose output through the liver. However, it still remains unknown if IL-6 directly increases endogenous glucose production or if a synergistic 'contraction factor' is required to increase HGO.

Recently, O'Neill et al. [172], tested the theory that IL-6 plays a role in glucose metabolism during exericse in an IL-6 knockout (KO) mouse model. Surprisingly it was found that IL-6 did not play a role in exercise induced skeletal muscle glucose uptake and no change in glucose homoeostasis was reported. Thus, the role of IL-6 in exercise mediated/induced glucose metabolism remains unclear.

The current study aimed to use an isolated liver model to test the effect of circulating IL-6 on hepatic glucose production in combination with glucagon, adrenaline or with human plasma separated from resting or exercised human blood.

Methods

Animal and human ethics

All animal and human experimentation were approved and conducted in accordance with the regulations adopted by the University of Auckland Animal and Human Ethics Committees.

Overview of isolated liver experiments

Two experiments were designed to test the effect of rhIL-6 on HGO in the isolated rat liver which are detailed below. Briefly, experiment 1 was designed to test the direct role of rhIL-6 of either high (100 pg/mL) or low (20 pg/mL) physiological concentrations on HGO with or without synergistic infusion of glucagon (1.15 nM) or adrenaline (50 nM) in an isolated rat liver. Experiment 2 was designed to test the role of human fasted or post-exercise plasma perfusion with or without synergistic rhIL-6 (20 pg/mL) infusion on HGO in an isolated rat liver.

Isolation and perfusion protocol of rat liver

Male Sprague-Dawley rats (170-190 g) were maintained up until the experiment on 12 h day-night cycle; water ad lib; and food ad lib (Harlan Teklad 2018 diet, Madison, WI, USA). Non-fasted rats underwent laparotomy under general anaesthesia (75 mg/kg body weight ketamine, 10 mg/kg body weight xylazine, intraperitoneal administration, i.p.). The liver was perfused *in vivo* and then removed (non re-circulating mode) in a similar manner to that previously reported [360].

Briefly, the portal vein was cannulated *in situ* and the atria vented to allow the liver to be perfused initially with 20 mL of heparinised perfusion media (vehicle) (final; NaCl 128 mM, MOPS 23.9 mM, KCl 6 mM , $MgSO_4$.7H2O 1.18 mM, CalCl2 1.29 mM, BSA(FFA) 0.2 %, pH 7.4). The *in situ* liver was then perfused at 2 $mL{\cdot}min^{-1}$ (connected to a custom- made, temperature-controlled organ perfusion system and perfusion medium oxygenated using carbogen, O_2: 95 %; CO_2: 5 %; 37°C) while the superior vena cava was cannulated and the liver excised. The liver was weighed and re-connected to the organ perfusion system whereby perfusion was continued at a rate of 2 $mL{\cdot}g^{-1}{\cdot}min^{-1}$ (of liver weight, $\sim$ 12 grams) with oxygenated and warmed perfusion medium (Refer to Figure 8.1 for diagram of the isolated liver system). The liver was stabilised for 40 min (t = 40). Organ effluent (1 mL) was then collected every 5 min for experiment 1 (detailed below) and every 1 min for experiment 2 (detailed below) for glucose measurement, using a GEM3500 glucose analyser (GEM Premier 3500, Instrumentation laboratory). Intra-assay correlation

coefficient of the GEM3500 glucose analyser was less than 0.5 %. Only male rats were used in the current study due to the possible effects of the menstrual cycle on metabolism.

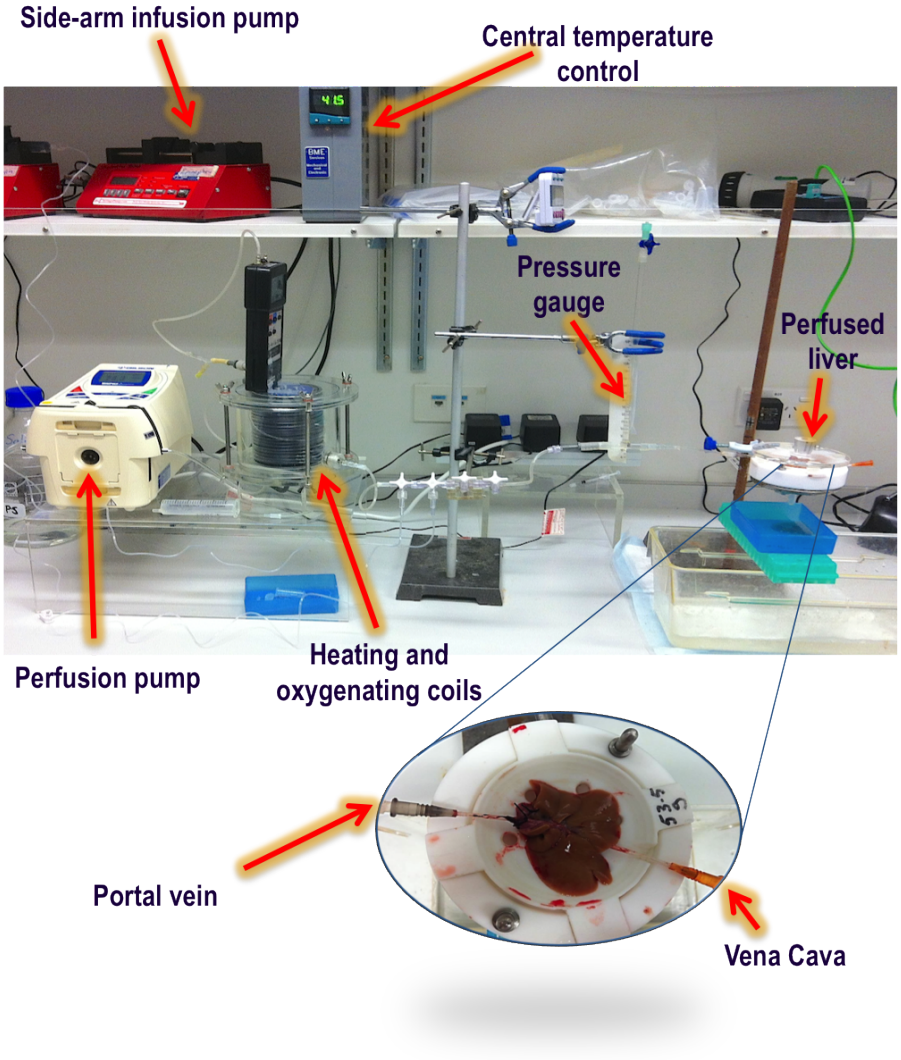


Figure 8.1: Diagram of the custom-made temperature-controlled organ perfusion system.

Isolated liver experiments

Experiment 1

High or low rhIL-6, glucagon and adrenaline perfusion

At t = 50 min, rhIL-6 20 pg/mL or rhIL-6 100 pg/mL was delivered via a side arm infusion (no side arm infusion in vehicle only trials). Post liver organ effluent (1 mL) was collected every 5 min for 15 min for glucose measurement using a GEM3500 glucose/gas analyser. At t = 65 min glucagon (1.15 nM) or adrenaline (50 nM)

was delivered through a second side arm. Post liver effluent (1 mL) continued to be collected every 5 min for 15 min for the measurement of glucose (see Figure 8.2 for an overview of the experimental design).

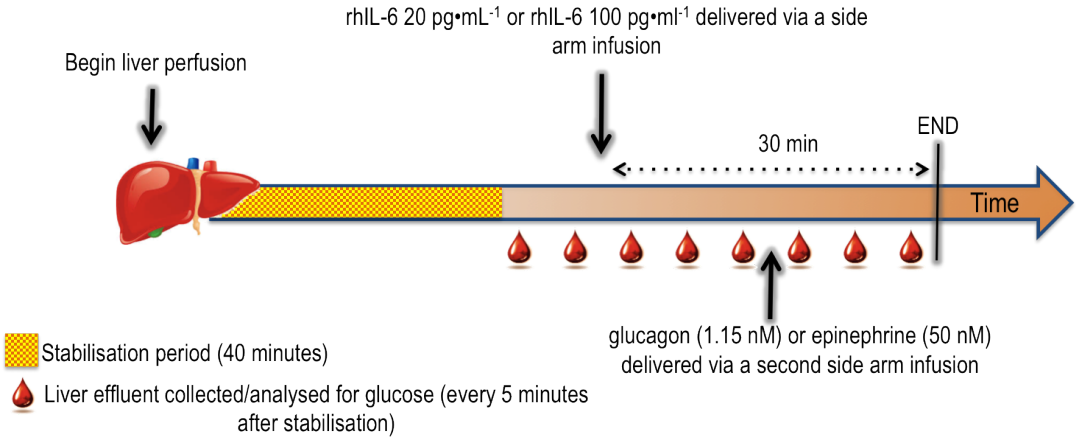


Figure 8.2: Overview of the perfusion protocol for experiment 1: synergistic infusion of high (100 pg/mL) or low (20 pg/mL) rhIL-6 and glucagon or adrenaline.

Experiment 2

Prior to liver perfusion for experiment 2, fasting and exercised human plasma was obtained via the following methods:

Participant

One healthy but untrained male (Age: 33 y, bodyweight: 90 kg, height: 185 cm) completed the exercise component of the current study.

Exercise protocol

The participant performed 3 separate trials of the exercise protocol designed and previously described by Meckel et al. [44] which was shown to significantly increase IL-6 above pre-exercise levels. Briefly, the participant completed a maximal 100 m run outdoors with the result (in $km \cdot h^{-1}$) subsequently used to calculate the speed at which the repeated-sprints during the exercise session would be performed (80 % of maximal 100 m speed). The participant completed the 100 m maximal sprint in 18.51 seconds at a speed of 19.46 km^{-1} and therefore completed all sprints of the exercise protocol at a speed of 16 $km \cdot h^{-1}$.

The participant performed a 5 min warm up on an electronically braked treadmill at 8 $km \cdot h^{-1}$ followed by a 1 min passive rest period. The participant then com-

pleted a decreasing distance repeated-sprint session on the treadmill consisting of single 400 m, 300 m, 200 m and 100 m sprints at 16 km·h^{-1}. Decreasing passive rest periods of 4 min, 3 min, and 2 min respectively separated sprints (Figure 8.3). All trials were performed at 08:30 h in the morning following an overnight fast. Exercise trials were separated by at least 7 days.

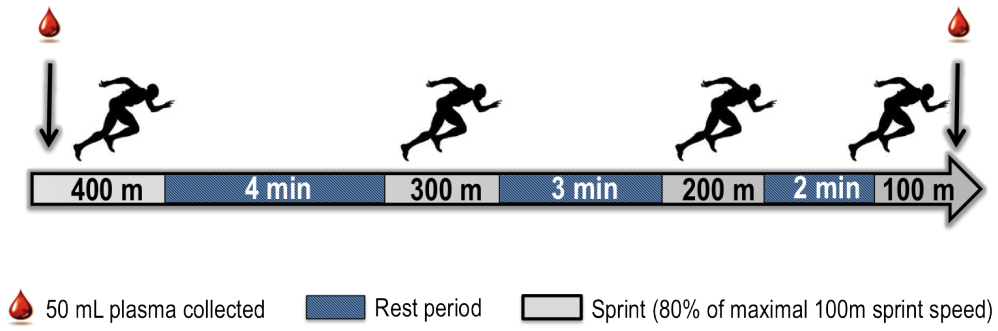


Figure 8.3: Overview of the repeated-sprint protocol employed as the exercise component for the current study.

Blood sampling

With the participant in a supine position, an indwelling venous cannula was inserted in to the antecubital vein. A 100 mL resting, fasted blood draw was taken immediately prior to the start of the exercise protocol. A second 100 mL blood draw was taken immediately following the exercise protocol. An extra 1 mL of blood was drawn pre and post-exercise for the immediate analysis of blood glucose and blood lactate using a portable epoc blood glucose monitor (epoc^{TM} Epocal, Inc.). Blood samples were immediately spun for 15 min at 4°C at 3000 rpm. Plasma was immediately separated and stored in separate (fasted and exercised) 50 mL containers at -80°C until required for the liver perfusion.

Human plasma and rhIL-6 perfusion

At t = 50 min rhIL-6 (20 pg/mL) or no rhIL-6 for control trials, was delivered via a side arm infusion. Organ effluent (1 mL) was then collected every 1 min for glucose measurement, using a GEM3500 glucose/gas analyser until the end of the experiment. Finally, at t = 55 min human plasma ((1 × concentrated, i.e. 25 mL of plasma diluted with 25 mL vehicle to equal the approximate concentration of plasma in whole blood) fasted or exercised plasma (50 mL total)) was then perfused at 2 mL·g^{-1}·min^{-1} for 5 min (collected (first pass) and re-perfused once (second pass); approximately 2.5 min for 50 mL of plasma to flow through from the start to the end

of the perfusion line) (see Figure 8.4 for an overview of the experimental design). Post liver plasma was collected every 1 min for glucose measurement and then frozen at -80°C.

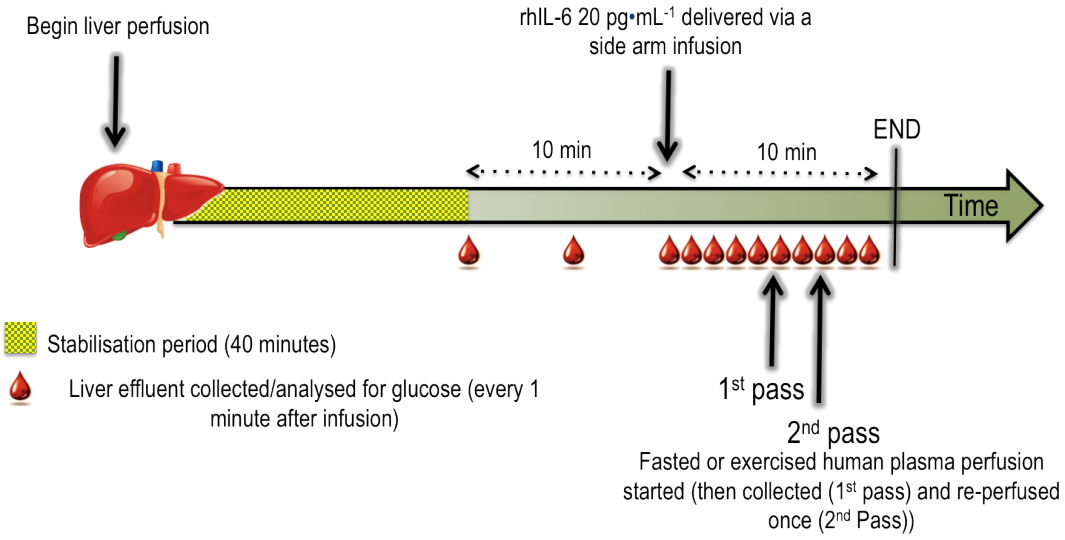


Figure 8.4: Schematic of the perfusion protocol for fasted or post-exercise human plasma with and without rhIL-6 infusion.

Analysis of plasma and perfusate

Plasma and organ effluent IL-6 concentrations were analysed using a high sensitivity human cytokine magnetic bead kit (MILLIPLEX MAP HCYTOMAG-60K). Plasma and perfusates were analysed for IL-6 according to the manufacturer's instructions except samples were analysed in singleton. Plates were analysed using the Luminex xMAP technology (Luminex Corporation). Intra-assay correlations of coefficient (CV) were less than 5 %.

Metabolic hormone analysis

Plasma concentrations of insulin and glucagon were analysed with a Milliplex MAP Kit human metabolic hormone magnetic bead panel (Cat HMHMAG-34 K) according to the manufacturer's instructions except samples were analysed in singleton. Plates were analysed using the Luminex xMAP technology. Intra-assay CV's were less than 5 %.

Statistical analysis

The area under the curve (AUC) for delta glucose output was calculated by trapezoid method for each of the conditions in both experiment 1 and experiment 2. In experiment 1, AUC was compared between all 3 conditions in both the glucagon and adrenaline trials by one-way ANOVA with Tukey's post hoc comparisons. In experiment 2, AUC was compared between the same condition with and without rhIL-6 by unpaired t test. Analysis of pre and post-exercise blood glucose and lactate concentrations were determined by paired t test. For all analyses differences were considered significant at a level of $p < 0.05$. All results are presented as means ± SD.

Results

Experiment 1

No significant effects were observed for rhIL-6 infusion of either 20 or 100 pg/mL on HGO compared with vehicle perfusion only (vehicle: 4.00 ± 0.38 vs. vehicle + IL-6(20 pg/mL): 4.25 ± 0.53 vs. vehicle + IL-6(100 pg/mL): 4.03 ± 0.34 μmol/g, $p = 0.45$).

The synergistic effect of rhIL-6 on HGO with either glucagon (Figure 8.5) or adrenaline (Figure 8.6) which are known to increase with exercise, was tested. In the glucagon experiment, a significant reduction in HGO AUC in both the 20 (vehicle + glucagon: 40.38 ± 1.93 vs. vehicle + glucagon + rhIL-6 (20 pg/mL): 35.38 ± 1.49 μmol/g, $p = 0.005$) and 100 (vehicle + glucagon: 40.38 ± 1.93 vs. vehicle + glucagon + rhIL-6 (100 pg/mL): 35.13 ± 1.49 μmol/g, $p = 0.0038$) pg/mL rhIL-6 groups compared with the vehicle and glucagon only group was observed (Figure 8.5). However, there were no differences in HGO AUC between the 20 and 100 pg/mL rhIL-6 trials, $p = 0.96$. Non-significant reductions in HGO AUC were observed when adrenaline was infused with 20 (vehicle + adrenaline: 20.88 ± 1.25 vs. vehicle + adrenaline + rhIL-6 (20 pg/mL): 18.13 ± 2.06 or 100 pg/mL (vehicle + adrenaline: 20.88 ± 1.25 vs. vehicle + adrenaline + rhIL-6 (100 pg/mL): 19.88 ± 1.109 μmol/g) of rhIL-6 compared with the vehicle and adrenaline only trials, and no differences were recorded between 20 or 100 pg/mL trials ($p = 0.084$) (Figure 8.6).

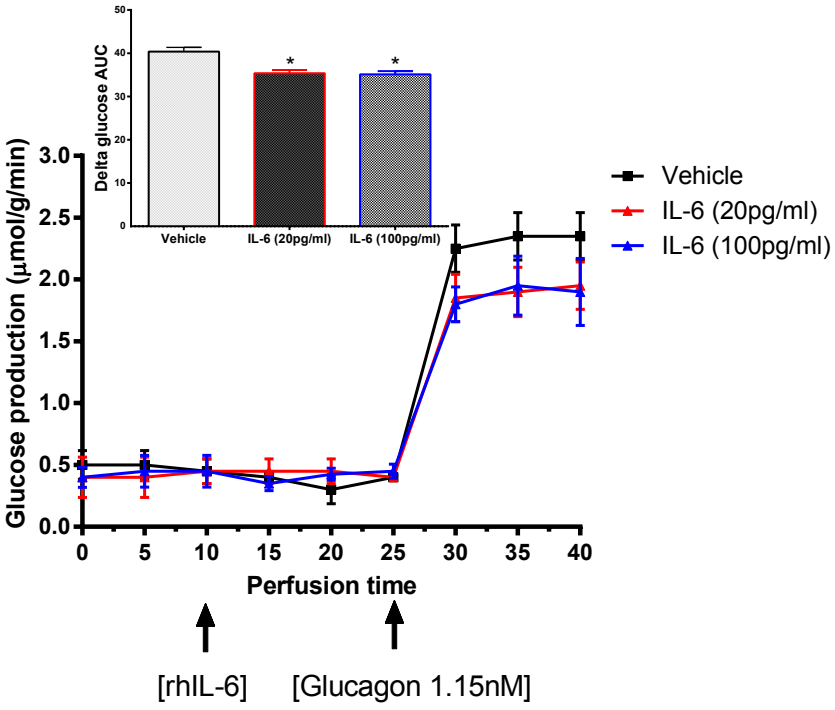


Figure 8.5: The direct effect of rhIL-6 (20 or 100 pg/mL) with or without synergistic infusion of glucagon on HGO in the isolated rat liver. *Significantly different to vehicle only trial, $p > 0.05$. Data are means ± SD.

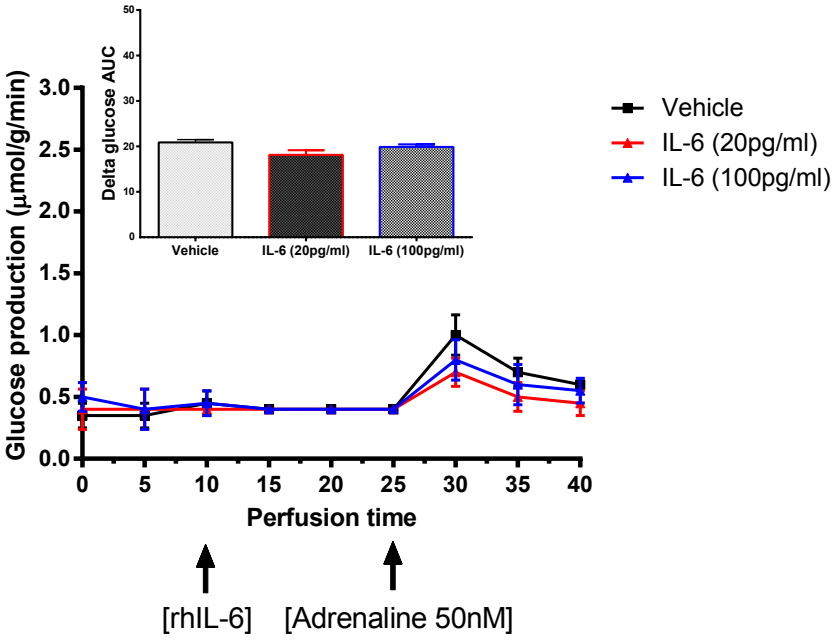


Figure 8.6: The direct effect of rhIL-6 (20 or 100 pg/mL) with or without synergistic infusion of adrenaline on HGO in the isolated rat liver. Data are means ± SD.

Experiment 2

A significant increase in blood glucose (pre-exercise: 5.43 ± 0.06 vs. post-exercise 6.07 ± 0.06 mmol/L, $p = 0.003$) and lactate (pre-exercise: 1.22 ± 0.22 vs. post-exercise: 17.90 ± 0.76 mmol/L, $p = 0.001$) in post-exercise blood compared to pre-exercise blood was observed (Figure 8.7). Significant increases in plasma IL-6 (pre-exercise: 4.44 ± 3.0 vs. post-exercise: 19.33 ± 2.48 pg/mL, $p = 0.032$), insulin (pre-exercise: 334.2 ± 61.56 vs. post-exercise: 773.2 ± 230.4 pg/mL, $p = 0.002$) and glucagon (pre-exercise: 21.27 ± 3.20 vs. post-exercise: 32.57 ± 4.38 pg/mL, $p = 0.000$) following the exercise protocol compared with pre-exercise plasma were also recorded (Figure 8.7).

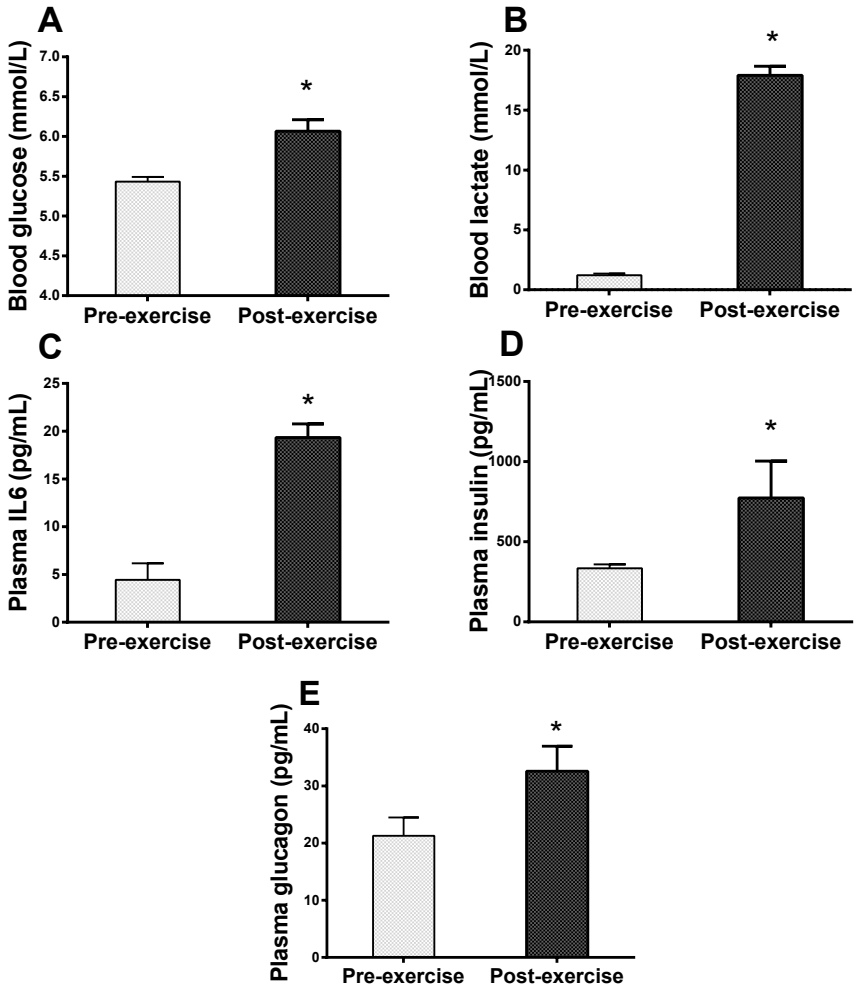


Figure 8.7: The effect of exercise on (A) blood glucose, (B) blood lactate, (C) plasma IL-6, (D) plasma insulin and (E) plasma glucagon. *Significantly different to pre-exercise concentration, $p < 0.05$. Data are means ± SD.

Non-significant reductions in AUC for HGO were recorded between vehicle and vehicle + rhIL-6 (vehicle: 6.63 ± 1.76 vs. vehicle + rhIL-6: 6.02 ± 1.17 μmol/g, $p = 0.65$) and post-exercise plasma and post-exercise plasma + rhIL-6 (post-exercise plasma: 16.36 ± 3.15 vs. post-exercise plasma + rhIL-6: 12.77 ± 1.74 μmol/g, $p = 0.16$) groups (Figure 8.8). When rhIL-6 was infused with fasting human plasma, a significant reduction in HGO AUC was observed compared to the fasting human plasma without rhIL-6 infusion (fasting plasma: 12.18 ± 1.17 vs. fasting plasma + rhIL-6: 9.10 ± 0.98 μmol/g, $p = 0.025$) (Figure 8.8).

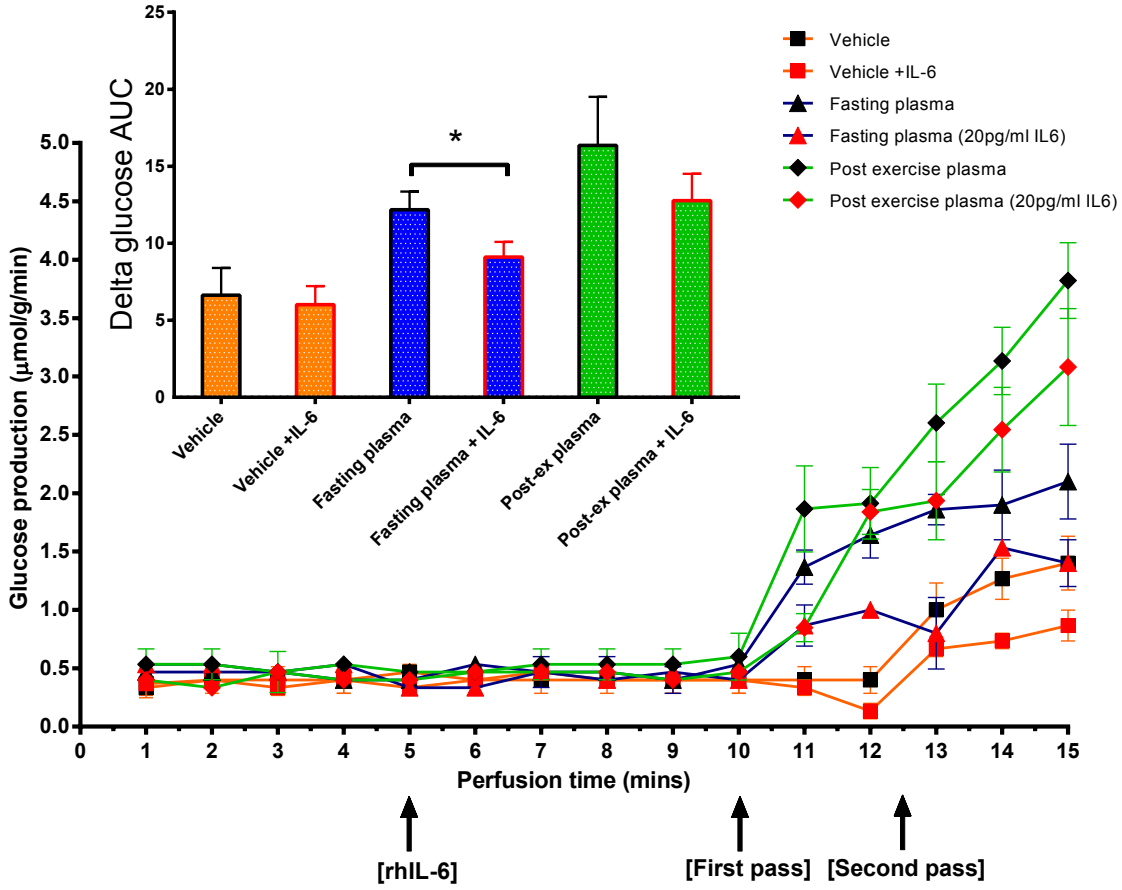


Figure 8.8: The direct effect of rhIL-6 (20 pg/mL) on HGO in the isolated rat liver with fasting and post-exercise human plasma. *Significantly different to concomitant fasting plasma only trial, $p < 0.05$. Data are means ± SD.

Discussion

It was demonstrated in the current study that rhIL-6 at physiological levels similar to those induced by exercise (moderate-high (20 pg/mL or high-extreme (100 pg/mL)) did not increase HGO in an isolated rat liver. Further, when rhIL-6 was infused with adrenaline no synergistic effects on HGO were recorded. However, when rhIL-6 was infused with glucagon, a significant reduction in HGO compared with glucagon infusion alone was observed. It has been suggested that an unknown 'contraction factor' may be involved in the synergistic regulation of HGO with IL-6 during exercise [35]. Therefore it was decided to test the 'contraction factor' theory via the perfusion of fasted non-exercised and exercised human plasma through an isolated rat liver. Interestingly, a significant reduction in HGO in the non-exercised plasma and a non-significant reduction in HGO in the exercised plasma when perfused with rhIL-6 in comparison to their control (no rhIL-6) trials was observed. For the first time, the current study was able to demonstrate that IL-6 may have a negative effect on HGO.

Large increases in lactate accompanied by increases in concentrations of glucose, glucagon and insulin were recorded immediately post-exercise and are indicative of metabolic stress during the exercise protocol employed by the current study. IL-6 was also significantly elevated immediately post-exercise which is commonly reported in human exercise studies [44, 137, 139, 145, 151, 218]. Therefore it appears likely that the exercise protocol in the current study would have been a sufficient stimulus for the co-secretion of possible 'contraction factor(s)' involved in the synergistic regulation of circulating glucose with IL-6. As rhIL-6 failed to produce an increase in HGO in resting or post-exercise human plasma, it is proposed that another 'contraction factor' may be responsible for the regulation of HGO during exercise.

The hypothesis that IL-6 may regulate HGO during exercise was based on previous observations that both the expression of IL-6 in human skeletal muscle and release in to the circulation have been shown to be related to skeletal muscle glycogen content [37] and exercise intensity [148]. As well, CHO ingestion has been shown to attenuate the exercise induced plasma IL-6 response [163]. However, the results of the current study suggest that this relationship may not result in a direct causal effect of circulating IL-6 on glucose production in the liver. There is however, the possibility that IL-6 produced within the skeletal muscle may have a different isoform to that used for infusion, or is bound to an IL-6 receptor when released. However, previous research has demonstrated that rhIL-6 is biologically active on tissues in sprague-dawley rats [361], but future research investigating the effects of bound and unbound IL-6 on HGO are warranted.

Training studies in healthy humans provide mixed results regarding changes in the circulating [200, 236, 237, 239] or local skeletal muscle [147, 155, 156, 240] IL-6 response to acute exercise after training, despite increases in performance likely associated with higher resting skeletal muscle glycogen and decreased glucose reliance during exercise. If IL-6 had a significant role in muscle to liver crosstalk with the purpose of altering the rate of glucose production by the liver during exercise, it would be expected that an attenuation in the IL-6 response alongside training induced changes in CHO metabolism may occur.

In support of the results of the current study, which suggest IL-6 does not increase endogenous glucose production, Helge et al. [245] reported that IL-6 release across the exercising arm or exercising leg during whole-body (90 min combined arm and leg exercise exercise) exercise was not correlated with glycogen utilisation or uptake of exogenous substrate in humans. It was also more recently shown using an IL-6 KO mouse model that maximal exercise capacity, body mass, energy intake and output, substrate utilisation and glucose and insulin tolerance did not differ between IL-6 KO and matched controls [172]. Following exercise (40 min at 70 % maximal running speed) plasma IL-6 was elevated in control mice only, to 39 pg/mL, while plasma glucose concentrations, $\dot{V}O_2$ and respiratory exchange ratio (RER) were not different between IL-6 KO mice and controls at the end of exercise. As well, skeletal muscle and glucose clearance were not different between groups [172], therefore, suggesting IL-6 does not influence glucose uptake in skeletal muscle or influence glucose homoeostasis during exercise. The results of the latter study were similarly reported in another recent IL-6 KO mouse model, where IL-6 appeared to have no effect on glucose homeostasis [362]. Interestingly, in this study IL-6 KO mice showed an impaired endurance running capacity at high relative velocities, however IL-6 KO mice showed lower oxygen cost of running at the same speed, and lower plasma lactate concentrations at submaximal and maximal running velocities compared to control wild type mice. These results thereby led the authors to suggest that IL-6 is not a major regulator of exercise capacity and appears unlikely to be requisite for glucose production during exercise.

In summary, it was shown that rhIL-6 did not increase glucose output from an isolated rat liver. Further, it was demonstrated that rhIL-6 had no synergistic effect with glucagon or adrenaline on regulating HGO. Most interestingly, acute rhIL-6 perfusion with human fasted or exercised plasma significantly and non-significantly reduced HGO, a direct contrast to current theory. For the first time, this study demonstrates that IL-6 may work as a negative regulator of HGO independent of an unknown 'contraction factor'.

CHAPTER 9

General Discussion

Introduction

The overall aim of the series of studies undertaken for this thesis was to investigate the efficacy of concurrently training repeated-sprint (RSA) and resistance (RT) exercise in order to improve team-sport performance variables in female team-sport athletes. Additionally the thesis also aimed to determine the physiological response to acute and chronic concurrent training in order to evaluate the compatibility of RSA and RT exercise adaptations. The thesis also aimed to determine the effectiveness of using inflammatory cytokines as a measure of exercise training load. An additional aim to determine the role of circulating IL-6 on hepatic glucose output (HGO) was added to the thesis following the findings of the preceding experimental chapters. The overall findings of the performance and physiological components of the thesis will be discussed and summarised as a cohesive whole, and areas for future research alluded to where appropriate. Limitations of the current research will also be discussed. Finally, practical applications of the research will be presented.

Effectiveness of concurrent RT and RSA exercise at improving team-sport performance variables

As RSA and power/strength qualities have been shown to be fundamental for team-sport athletes [6–8, 11–13], the improvement of these qualities through structured concurrent training were considered throughout this thesis.

Intra-session exercise sequence did not affect the ability to maximally perform the second mode of exercise (Chapter 5), with no signs of lasting peripheral fatigue evident in power outputs during squats or sprints when these were performed last. There were also no differences recorded in rating of perceived exertion (RPE) during RT and RSA exercise irrespective of the order in which they were undertaken. Interestingly, these findings differ to previous literature involving more traditional aerobic exercise, which has been shown to cause reductions in force production or squat performance, during a subsequent resistance session, albeit with different performance measures [304, 309].

Four weeks of concurrent RT and RSA training significantly increased all maximal strength, power and speed variables in trained female team-sport athletes irrespective of training structure. As there were no obvious changes in molecular proteins associated with protein synthesis, satellite cell hypertrophy, or significant increases in thigh girth following training (Chapters 6 and 7) it appears that molecular adaptation and increases in muscle size did not predominate the adaptive processes required to increase these performance parameters. Instead, improvements could have been due to other factors, such as: neuromuscular adaptations, changes in fibre type/myosin isoforms and/or mechanical/technique adaptations that result in improved kinematics (task-specific learning). Previous research supports a greater neural contribution to strength gains in the early phases of strength training [85, 363]. Increases in EMG amplitude have been shown to occur before changes in muscle size [85, 364]. These increases may be attributed to increases in motor unit firing rate or synchronisation of fibre recruitment [365, 366]. Practical outcomes of increased neural input can include increases in contraction velocity, increases in contraction force or both, resulting in a greater whole muscle power output [364, 367].

Myosin isoform composition of skeletal muscle plays a major role in the contractile characteristics, such as velocity of shortening and maximal power output [368]. Changes in these compositions may represent important adaptations during training. Resistance training [369–371], sprint training [372] and concurrent training [373] have all been shown to be able to alter myosin isoform composition towards type II muscle fibres. However, these studies were over longer training periods and it is difficult to determine whether the duration of the current research (Chapter 6 and 7) was long enough for significant transformations to take place.

Neural adaptations may contribute to the ability to significantly increase the load lifted during initial strength training and the subsequent overload stimulus in which the muscle would be subjected. As training continues, an increased load may further maximise strength gains through morphological/molecular modifications. Future research could determine when adaptation changes predominantly from neuromuscular improvements to alterations in muscle size and architecture

in trained females completing concurrent exercise similar to that prescribed in the current thesis.

Another factor that could have resulted in increased sprint performance is improved intramuscular coordination that can lead to mechanical and/or kinematic adaptations, such as increases in stride frequency or length [374]. Anecdotally, it was noted that some athletes were actively considering their sprint technique and thus self-learning leading to mechanical adaptations in gait or stride frequency may have occurred. In support of this, it has been previously shown that faster accelerating (over 15 m) field-sport athletes have shorter left and right foot ground reaction times which translate to greater stride length compared to slower accelerators [349].

Though not analysed in detail in this thesis, it would have been interesting to divide the shuttle sprint in to 3 components; acceleration, deceleration and change of direction (COD) (180 degree turn). This may have enabled a greater understanding of what aspect of performance during the sprint was improved with training that resulted in a faster total sprint time during the repeated-sprint session and greater maximal linear 10, 20 and 30 m sprint performances (Chapter 6 and 7). For example, there may have been increases in initial acceleration, reductions in the time needed to decelerate prior to turning, and/or an improvement in the ability to change direction as a consequence of both increases in leg strength from the resistance training [375] and specificity of movement during the shuttle sprints [348]. The ability to change direction while sprinting is important to many team-sport based athletes [376], and it has been shown to distinguish between elite and sub-elite soccer players [377]. Both strength training and specific COD training have been shown to improve this component but the most effective method of training is still to be established [376]. Thus, specific sprint technique coaching with particular specificity to the movements performed during match-play may be beneficial to team-sport athletes.

Repeated sprint protocols with longer duration sprints ($>$ 15 s) have been shown to increase $\dot{V}O_2$max [28,128], however this training effect was not exhibited in this thesis (Chapter 7). This is likely due to the shorter duration of the sprint efforts ($\sim$ 5 s). Aerobic capacity, as discussed in Chapter 2, is also important for team-sport athletes [1, 7, 110, 118] and therefore for some athletes it may be desirable to also improve rather than just maintain this fitness quality depending on the season phase and current fitness level. Repeated-sprint protocols with longer duration sprints ($\sim$15-20 s with up to 1-4 min rest periods) have elicited improvements in aerobic capacity [28, 128, 378], likely due to the greater oxidative contribution to sustain the sprint efforts.

However, had longer distance/duration sprints been performed within the repeated-sprint bout of the current research, total time spent sprinting would have increased, and may have created a trade-off between improving $\dot{V}O_2$max, and im-

proving strength and power development. Longer duration sprints may increase the potential for acute residual peripheral fatigue, possibly due to greater reductions in muscle glycogen content [118] or muscle damage which may reduce the ability to produce tension in the skeletal muscle during the resistance protocol [304]. While there was no evidence of acute residual fatigue within the current RT:RSA design, there may be the potential for the intra-session ordering to be of more importance if a greater aerobic component was to be introduced into the sprint efforts, and would therefore need to be trialled. Also, repeated-sprints with a greater aerobic contribution may be more likely to cause interference with the molecular adaptation to resistance exercise [129, 275]. Although the results of the current thesis suggest that molecular adaptation likely did not contribute considerably to improved performance (Chapter 6) following 4 weeks of concurrent training (possibly due to neuromuscular factors being the predominant adaptations during the initial phase of training) a longer training period may have resulted in an increase in the contribution to strength gains by molecular adaptation and muscle hypertrophy [85]. It was observed that cumulative glucose and cortisol responses were somewhat attenuated following training suggesting lowered blood glucose reliance post training and improved RSA exercise tolerance, however this did not translate to an improved fatigue index during the RSA protocol.

In summary, combining the performance and corresponding physiological data from the series of experimental studies contained within this thesis, suggests that little acute or adaptive interference during acute and prolonged concurrent RT and RSA exercise training appears to exist. Therefore, it seems that the adaptations involved in improving both repeated-sprint and maximal strength performance are compatible, providing support for the inclusion of concurrent RSA and RT training within team-sport training programmes. However, increasing our understanding of the underlying adaptations responsible for improvements in performance in trained female athletes may help to specify training further. It may be possible to explicitly target these adaptations to provoke more substantial and/or a greater rate of performance gains.

Can cytokines be used as a measure of training load?

Previous literature has suggested that exercise induced alterations in inflammatory cytokines and hormones of the growth hormone↔insulin-like growth factor-1 (GH↔IGF-1) axis could be used as a potential measure of training load [43, 44]. The relative ease with which cytokine and hormonal responses can be measured (via blood draw and analysis) would suggest they could be acceptable candidates for measuring long-term training load over a wide range of exercise modes. This

would provide the ability to measure training load of different modes of exercise that are not easily compared using simple standard physiological measures (HR, RPE). However, these previous investigations have focused on acute exercise protocols and hence their acute physiological response only, and were therefore limited in their ability to determine the efficacy of these bio-markers as a measure of long-term training load. The current body of experimental studies aimed to provide insight into the responses of these bio-markers from both an acute and chronic training perspective in order to provide a more robust assessment of the effectiveness of using these bio-markers as a practical measure of training load.

The prescribed acute single-mode RT or RSA exercise was not shown to be a sufficient stimulus to elevate the cytokines or endocrine hormones above pre-exercise levels (Chapter 4), interestingly though, both HR and RPE were elevated above rest during RT and RSA exercise suggesting these simple measures may be more sensitive markers of training load, at least within exercise modes. However, once the exercise modes were combined (Chapter 5), significant and immediate post-exercise elevations in both cytokines and endocrine hormones were observed, confirming that duration and intensity play an important role in these responses as previously reported [31, 32]. In the current experimental studies, a clear relationship between inflammatory cytokines and endocrine hormones was not observed (as discussed previously in Chapter 4), and suggests that perhaps systemic IL-6 concentrations greater than ∼80 pg/mL may be required to stimulate increases in cortisol. Further investigations into the relationship between the neuroendocrine and inflammatory cytokine response during exercise may be beneficial to increase our understanding of the interplay between these two systems.

If cytokine and hormonal responses are reflective of exercise training load, as suggested by Meckel et al. [43,44], the above findings would suggest that performing the prescribed exercise concurrently in the same session should result in a greater training overload stimulus, potentially leading to greater training adaptations. The results of Chapters 6 and 7 did not show any significant differences in performance improvements between the two training groups (same session/alternating day) at the end of a four-week training period. This may indicate that the acute cytokine and endocrine responses played only a trivial role in the adaptive processes that led to improved performance. Therefore, it may be difficult to use these markers as measures of training load if they are not involved in the adaptation to the exercise.

Interestingly, it was observed that there were comparable elevations in circulating and local cytokines after heavy and maximal exercise before and after 4 weeks of concurrent RT and RSA training (Chapter 7). Previous studies, investigating both plasma [379] and skeletal muscle [155, 156] cytokines have observed increases in circulating and/or local cytokines to acute exercise that were not attenuated by phases

of resistance [155, 156] or endurance [379] exercise training. As others have failed to show an attenuation of the cytokine response following training periods longer than that of the current study it is therefore possible that the systemic and local cytokine responses to concurrent RT and RSA exercise are not (easily) altered by team-sport specific concurrent training. The failure to attenuate cytokine responses with training, suggests that inflammatory cytokines may have an essential role to play in the immediate post-exercise recovery period. Accordingly, while others have reported that exercise training [155, 156] or training status [379] does not significantly alter the cytokine signalling mileu to acute exercise, no mechanisms have yet been proposed to explain physiologically why these signalling proteins are unaltered by training, other than that they are likely essential to the acute adaptation to exercise.

As the cytokine response is often thought to be associated with inflammation related to acute muscle damage/disruption [34, 134, 380], one may expect the inflammatory response to be affected by the repeated bout effect (initial bout of damaging exercise protects against subsequent damage during similar bouts of exercise [381]). It would then be expected that with training and accustomisation to exercise, resulting muscle trauma would be blunted, and the inflammatory cytokine response would be attenuated accordingly [381]. The current body of work and previous investigations do not necessarily support this response. Though, it must also be acknowledged that the kinetics of the early and prolonged cytokine response may be differently affected by training, and had the current thesis (and previous studies) measured beyond 2 h and in the days following exercise, an attenuation in the response may have been observed.

It is possible that a minimum inflammatory response (dependent on the combination of duration and intensity of the contractile stress) is essential to maintain the integrity of the skeletal muscle after mechanical loading. Either way, the underlying mechanisms for the cytokine response following concurrent exercise is likely multi-factorial and may include internal input from neuroendocrine, metabolic and immune factors. Therefore, if inflammatory cytokines are indeed critical for maintaining and/or assisting the structural repair and acute adaptive responses of skeletal muscle after exercise, it may be requisite for these cytokines to remain responsive to exercise regardless of training status. Future research is recommended to determine the responsive role of these cytokines to exercise and define what their essential role in the immediate post-exercise recovery period may be. Until we know more about the role the inflammatory cytokines play in response to exercise there would appear to be little value in using them as a measure of training load and/or stress.

IL-6 and hepatic glucose output

As discussed in previous chapters of this thesis, IL-6 is thought to play a role in the regulation of glucose metabolism during exercise [33,35,382]. One way in which circulating IL-6 is hypothesised to do this, is through cross-talk with the liver to increase hepatic glucose output (HGO) [33,159]. Throughout the course of this work, it became important to test the role of IL-6 directly on the liver, to support whole-body human studies that provided evidence for this current theory. Specifically, in Chapter 7 it was shown that 4 weeks of concurrent RT and RSA exercise training had no effect on the plasma IL-6 response to acute exercise despite a lowered blood glucose and cortisol response. No changes in IL-6 protein abundance in skeletal muscle occurred following acute concurrent exercise, suggesting that the skeletal muscle was not the predominant source of plasma IL-6. In Chapters 4, 5 and 7 it was also observed that while plasma IL-6 was elevated immediately post-exercise, peak plasma IL-6 concentration did not occur until 120 and 180 min in to recovery. Therefore, it was difficult to interpret the immediately post-exercise IL-6 response as it is possible that it could have been due to either metabolic or inflammatory mechanisms or that these mechanisms were working together simultaneously.

The primary focus of Chapter 8 was to investigate the physiological importance of circulating IL-6 on HGO. Chapter 8 used a novel isolated rat liver model to investigate this theory, and surprisingly it was shown that infusion of recombinant human (rh) IL-6 (20 or 100 pg/mL) did not increase HGO from an isolated liver. It was also demonstrated that rhIL-6 did not work in synergism with adrenaline to further increase HGO, and when infused with glucagon, HGO was surprisingly reduced. Similarly, when rhIL-6 was infused with human fasted and exercised plasma, a significant and non-significant reduction in HGO was observed. While this study represents pilot work, and has methodological limitations, it provides some evidence to suggest that the current understanding of one of the proposed roles of IL-6 during exercise is perhaps not as clear as first thought when this thesis began. For the first time, it was demonstrated that IL-6 may work as a negative regulator of HGO independent of an unknown 'contraction factor', a surprising contrast to popular theory [33,35,159]. Therefore, further research is required to confirm the role of IL-6 in regulating blood levels of glucose, which may in turn make the interpretation of the immediate post-exercise IL-6 response easier. As a consequence of the findings from this proof of concept study, the clinical and or physiological significance of the carbohydrate induced effects on the circulating IL-6 concentration during exercise requires further research.

The results of this thesis suggest that the immediate post-exercise systemic and local cytokine responses are not easily attenuated or modified by short-term training and may therefore be a fundamental element of the adaptive or homoeostatic responses to intense exercise. Accordingly, the cytokine response to exercise is a widely researched area, but the specific role of these proteins in the inflammatory process involved in the muscle damage, repair and remodelling cycle after exercise during the immediate recovery period remain to be understood. Until the physiological consequences of the inflammatory cytokine cascade initiated by exercise and the ability of this response to be altered by training are fully identified, it is premature to recommend inflammatory cytokines to strength and conditioning and sport science practitioners as a reliable bio-marker of training load to familiar exercise in well-trained athletes or of adaptation to exercise post-training. While there are limitations in the use of traditional and modified RPE and HR measures for the monitoring of internal training load as discussed in Chapter 3, RPE in particular, appeared to be more sensitive than alterations in inflammatory cytokines to changes in exercise intensity following 4 weeks of concurrent RT and RSA exercise within the current research. The particular sensitivity of RPE to the change in absolute exercise intensity during squat exercise following training suggests that athletes have an accurate subjective understanding of the physical strain experienced during exercise, how accurately reflective this is of actual internal physiological stress is difficult to determine from the current results. However, along with their particu-

lar ease of measurement and cost effectiveness and practicality within a team-sport training environment (large number of athletes), HR and RPE measures may be recommended for use of acute training load assessment above inflammatory cytokines at the present time. However, HR and RPE measures are limited in their ability to convey information about the metabolic and muscular stress of exercise and the possible required repairative processes. Therefore, it is suggested that research continue to investigate the use of bio-markers for measures of internal training load at this may provide greater insight into continuing physiological stress after the cessation of exercise. Greater mechanistic understanding of the role of cytokines following exercise may increase their value as a biological marker of exercise stress/training load in the future.

Limitations:

As with any research approach there are always going to be advantages and disadvantages of methodology and factors that if measured, could have added to the research findings. As such, what I regard as the main limitations of the thesis are discussed below.

Overall

- Due to both the cost of cytokine measurement, the amount of time and associated practicalities of keeping participants in the laboratory in to the night (or bringing them back in to the laboratory on multiple occasions), cytokine responses were only measured in the 2-3 h following exercise. However, the immediate post-exercise period would be a practical and accessible time for practising sport scientists to retrieve blood samples (while controlling for confounding variables such as nutrition) from athletes and thus this period was chosen for the current research.

Chapter 4

- As it was considered to be of interest to ascertain whether serum and plasma cytokine levels are comparable, only 5 participants acute exercise cytokine responses were measured as a consequence of allocating resources to plasma/serum samples. Had cytokine responses been measured in a greater n, subtle differences in cytokine concentrations following exercise may have been detected or may have allowed for the identification of possible 'high and/or low responders'.

Chapter 5

- An n of 8 was included in this experimental study. Due to the cost of the biological analysis, and the narrow population to be tested (female, practising trained team-sport athlete, taking a combined oral contraceptive of specific estrogen and progesterone make up) this number was deemed practical. Had a greater n been possible, there may have been the option of investigating whether there are genetically different profiles in the cytokine or hormonal response to exercise identified as 'high and/or low responders'.

Chapter 6 and 7

- In Chapter 6 and 7, an n of 6 for each training group was included. It is acknowledged that this n is small but was dictated by a number of factors associated with the medical care during and post the muscle biopsy procedure, laboratory space, training time and the population in which participants were sampled (female, practising trained team-sport athlete, taking a combined

oral contraceptive of specific estrogen and progesterone make up). Studies with similar sample sizes are available in current literature.

- In the current research the effect of same day training (SDT) or alternating day training (ADT) on performance outcomes was evaluated. A limitation of this research study was that a resistance only and repeated-sprint only training group was not included in which to compare the effectiveness of concurrently training these exercise modes with training them in isolation. However, team-sport athletes are required to perform strength, power and repeated-sprint type movements simultaneously throughout match-play and it was therefore decided that the SDT and ADT concurrent training groups would provide the most practical information within the context of the thesis aims.
- Muscle biopsies were taken at 60 and 180 min post-exercise in Chapter 7 which was based on both previous literature and personal experiences of the laboratory where skeletal muscle analysis was undertaken. The timing of peak signalling may have been missed in Chapter 7 due to gender and/or training status differences which may have been limiting when interpreting the signalling response to concurrent RT and RSA exercise, although this is speculative and further research is warranted.
- The post-training acute exercise protocol was performed at both the same absolute load (squat exercise), and same relative intensity (maximal repeated-sprints) which could have had the potential to mask small attenuation of physiological responses following the 4 week period of exercise training [240].

Chapter 8

- There are physiological differences between endogenous human and rat IL-6, however rhIL-6 has previously been reported to be active on rat cells [361]. There are also physiological differences in the HGO of humans and rodents and therefore may limit the transference of results.
- Due to the 'proof of concept' nature of the study, only plasma from 1 human participant was perfused through 6 different rodent livers and it is therefore difficult to generalise the results of this research to a larger population.

Future research:

The main findings from this thesis have shed light on the effectiveness of concurrent training specifically in female team-sport athletes, and the problems involved in measuring internal training load by alterations in inflammatory cytokines. However, in the process of arriving at these findings this thesis has also highlighted a number of areas that require further research.

Performance research

- Increasing our understanding of the underlying adaptations (molecular, neuromuscular, physiological, kinematic, skill/learning based adaptations) responsible for improvements in performance in trained athletes to concurrent RT and RSA exercise may help to specify training further. Investigations between males and females may also provide specific information for strength and conditioning practitioners when developing programmes for these populations.
- An interesting progression from the current research would be to determine how to adapt the current concurrent training protocol for long-term periods, to allow maintenance of the performance gains throughout the in-season. This may include sport specific actions being incorporated within the protocol, i.e., sprinting with the ball, tackling at the end of a sprint, jumping for a high ball while turning etc.
- A progression from the training study (Chapter 6 and 7) completed in this thesis would be to complete a time-motion analysis of match-play performance prior to and following the concurrent RT and RSA training to determine if the improvements in laboratory based team-sport performance variables translates in to significant and practical match-play performance.

Physiological research

- In Chapter 4, differences in detectable levels of inflammatory cytokines were measured in serum and plasma components of blood, similar to previous reports in non-exercising individuals [210]. It has also been shown that there may even be differences in detectable levels of cytokines between serum and plasma with different anti-/coagulants [383]. A methodological investigation of the most appropriate blood collection methods for optimal detection of cytokines in healthy humans exposed to exercising conditions would be a logical progression of the current research and be useful to encourage the standardisation of blood collection methods and comparability of results.
- An important question that should be answered in future research is whether the immediately post-exercise induced changes in cytokine concentration and white blood cell mobilisation is of clinical/physiological significance.
- Future studies should also aim to investigate the smallest worthwhile change (relevant to an identification of the physiological role of this response) in cytokine concentration following exercise; this may be dependent on the individual roles of each cytokine and the cause for which the cytokine has been elevated i.e., exercise, inflammation, metabolism, immune. As it currently

stands, interpretation of the post-exercise inflammatory response is challenging.

- In the current research, the peak signalling response to exercise may have been missed due to the timing chosen for the muscle biopsy. While previous research was used to help determine the timing of the muscle biopsy, the sex and or training status of the participants in the current research may have impacted the post-exercise signalling response. Therefore, investigations into the timing of skeletal muscle biopsies for particular proteins between sexes and participants with different training status are warranted to ensure optimal timing for future studies.
- Future concurrent resistance and repeated-sprint studies should aim to include nutritional interventions as the addition of protein supplementation prior to and following resistance [384], repeated-sprint [385] and 'traditional' concurrent exercise [386] has shown to stimulate post-exercise signalling and protein synthesis above exercise alone. Nutritional intervention following concurrent repeated-sprint and resistance exercise may increase the anabolic potential of the exercise that may result or contribute to greater improvements in maximal strength and/or power performance.
- The use of multiplex assays that utilise Luminex technology to measure protein concentrations of skeletal muscle cytokines is a relatively new technique and therefore the methodology of this process may yet to be optimised. It is possible that readings of cytokine concentration in skeletal muscle may be affected by the presence of blood within the sample. It could be suggested that future studies consider adding a whole blood control (after calculation of how much blood is present per mg of muscle) within one of the wells of the plate in which to normalise skeletal muscle sample concentrations against.
- Future studies are encouraged to progress the findings of Chapter 8. Octreotide acetate is a synthetic pharmacological derivative of naturally occurring somatostatin and its physiological activities include inhibition of glucagon, insulin and growth hormone release [387]. By replicating the isolated liver investigation of Chapter 8, the added step of administering a physiological dose of octreotide to the participant prior to exercise will inhibit glucoregulatory hormone control over HGO. This may allow greater determination of the isolated direct effect of IL-6 on HGO and may provide further confirmation of the direct role of circulating IL-6 on HGO.

Conclusions and practical applications

Overall the findings of this thesis suggest that there are commonalities between the initial adaptive responses to maximal running repeated-sprints and high-load, low-rep lower-body resistance exercise. It is likely that these changes are initially predominantly neuromuscular and/or mechanical in nature in female team-sport athletes familiar with RT and RSA. Further, it appears that total volume of concurrent RT and RSA exercise is more important than the volume of the acute stimulus and structure of concurrent training (SDT or ADT) for improvements in team-sport specific performance. There is practical evidence for the inclusion of a short (4 week) concurrent RT and RSA exercise training programme during the pre-season in order to improve preparedness for in-season training and match-play performance in female team-sport athletes.

From the findings of this thesis it appears that the cytokine response to exercise is dependent on the combination of exercise duration and intensity. However, concurrent RT and RSA training does not significantly alter the cytokine response to acute concurrent RT and RSA exercise in trained female team-sport athletes. This suggests that inflammatory cytokines may play an essential role in the immediate post-exercise recovery and adaptive period. This thesis also provides information that the role of circulating IL-6 in glucose metabolism is unclear, and that IL-6 may negatively regulate HGO at rest and during exercise, a contrast to current theory [33, 159, 359]. Therefore, until the physiological role of the inflammatory cytokines elevated by acute exercise but unattenuated by chronic training are more clearly understood, they are unlikely to be an informative measure of long-term training load for sport science practitioners.

Therefore, based on the findings of this thesis, sport scientists and strength and conditioning practitioners wanting to improve their team-sport based athletes' strength and RSA in the pre-season period may find the following recommendations of benefit.

- 4 weeks of concurrent RT and RSA exercise is an effective short-term training programme for inducing large improvements in performance parameters previously shown to relate directly with team-sport match performance.
- The structure of the 4 week concurrent training (within the same session or on separate days) or the order in which the two modes of exercise are performed do not appear to be overly important within a trained female team-sport population, instead total volume appears to be the defining factor. Strength and conditioning practitioners can therefore feel comfortable prescribing concurrent RT and RSA in a structure that is most convenient for the athlete without adverse effects on performance gains.

- Based on the current evidence provided by the thesis, it is not yet worthwhile for sport science practitioners to invest in cytokine monitoring in team-sport athletes for the measurement of training load.

References

[1] J. Bangsbo, M. Mohr, and P. Krustrup, "Physical and metabolic demands of training and match-play in the elite football player," *J Sports Sci*, vol. 24, no. 7, pp. 665–674, 2006.

[2] B. Cunniffe, W. Proctor, J. S. Baker, and B. Davies, "An evaluation of the physiological demands of elite rugby union using global positioning system tracking software," *J Strength Cond Res*, vol. 23, no. 4, pp. 1195–203, 2009.

[3] A. Davidson and G. Trewartha, "Understanding the physiological demands of netball: a time-motion investigation," *Int J Perform Anal Sport*, vol. 8, no. 3, pp. 1–17, 2008.

[4] D. Matthew and A. Delextrat, "Heart rate, blood lactate concentration, and time-motion analysis of female basketball players during competition," *J Sports Sci*, vol. 27, no. 8, pp. 813–21, 2009.

[5] S. E. McInnes, J. S. Carlson, C. J. Jones, and M. J. McKenna, "The physiological load imposed on basketball players during competition," *J Sports Sci*, vol. 13, no. 5, pp. 387–97, 1995.

[6] M. Mohr, P. Krustrup, and J. Bangsbo, "Match performance of high-standard soccer players with special reference to development of fatigue," *J Sports Sci*, vol. 21, no. 7, pp. 519–28, 2003.

[7] M. Spencer, S. Lawrence, C. Rechichi, D. Bishop, B. Dawson, and C. Goodman, "TimeÐmotion analysis of elite field hockey, with special reference to repeated-sprint activity," *J Sports Sci*, vol. 22, no. 9, pp. 843–850, 2004.

[8] J. Bangsbo, F. M. Iaia, and P. Krustrup, "Metabolic response and fatigue in soccer," *Int J Sports Physiol Perform*, vol. 2, no. 2, pp. 111–27, 2007.

[9] D. Austin, T. Gabbett, and D. Jenkins, "The physical demands of super 14 rugby union," *J Sci Med Sport*, vol. 14, no. 3, pp. 259–63, 2011.

[10] J. Lythe and A. E. Kilding, "Physical demands and physiological responses during elite field hockey," *Int J Sports Med*, vol. 32, no. 7, pp. 523–8, 2011.

[11] E. Rampinini, D. Bishop, S. M. Marcora, D. Ferrari Bravo, R. Sassi, and

F. M. Impellizzeri, "Validity of simple field tests as indicators of match-related physical performance in top-level professional soccer players," *Int J Sports Med*, vol. 28, no. 3, pp. 228–35, 2007.

[12] J. Hoff and J. Helgerud, "Endurance and strength training for soccer players: physiological considerations," *Sports Med*, vol. 34, no. 3, pp. 165–80, 2004.

[13] D. G. Baker and R. U. Newton, "Comparison of lower body strength, power, acceleration, speed, agility, and sprint momentum to describe and compare playing rank among professional rugby league players," *J Strength Cond Res*, vol. 22, no. 1, pp. 153–8, 2008.

[14] M. S. Chelly *et al.*, "Relationships of peak leg power, 1 maximal repetition half back squat, and leg muscle volume to 5-m sprint performance of junior soccer players," *J Strength Cond Res*, vol. 24, no. 1, pp. 266–71, 2010.

[15] M. Lopez-Segovia, M. C. Marques, R. van den Tillaar, and J. J. Gonzalez-Badillo, "Relationships between vertical jump and full squat power outputs with sprint times in u21 soccer players," *J Hum Kinet*, vol. 30, pp. 135–44, 2011.

[16] U. Wisløff, C. Castagna, J. Helgerud, R. Jones, and J. Hoff, "Strong correlation of maximal squat strength with sprint performance and vertical jump height in elite soccer players," *Br J Sports Med*, vol. 38, no. 3, pp. 285–288, 2004.

[17] K. Häkkinen *et al.*, "Neuromuscular adaptations during concurrent strength and endurance training versus strength training," *Eur J Appl Physiol*, vol. 89, no. 1, pp. 42–52, 2003.

[18] R. C. Hickson, "Interference of strength development by simultaneously training for strength and endurance," *Eur J Appl Physiol Occup Physiol*, vol. 45, no. 2-3, pp. 255–63, 1980.

[19] W. J. Kraemer *et al.*, "Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptations," *J Appl Physiol*, vol. 78, no. 3, pp. 976–89, 1995.

[20] J. P. McCarthy, J. C. Agre, B. K. Graf, M. A. Pozniak, and A. C. Vailas, "Compatibility of adaptive responses with combining strength and endurance training," *Med Sci Sports Exerc*, vol. 27, no. 3, pp. 429–36, 1995.

[21] M. Leveritt, P. J. Abernethy, B. K. Barry, and P. A. Logan, "Concurrent strength and endurance training. a review," *Sports Med*, vol. 28, no. 6, pp. 413–27, 1999.

[22] P. L. Wong, A. Chaouachi, K. Chamari, A. Dellal, and U. Wisløff, "Effect of preseason concurrent muscular strength and high-intensity interval training in professional soccer players," *J Strength Cond Res*, vol. 24, no. 3, pp. 653–60, 2010.

[23] K. Tsimahidis, C. Galazoulas, D. Skoufas, G. Papaiakovou, E. Bassa, D. Patikas, and C. Kotzamanidis, "The effect of sprinting after each set of heavy resistance training on the running speed and jumping performance of young basketball players," *J Strength Cond Res*, vol. 24, no. 8, pp. 2102–8, 2010.

[24] J. Alonso and G. Dias-Johnson, "The effects of six weeks of in-season specific

training on young swedish division 2 basketball players." Master's thesis, The Swedish School of Sport and Health Science, 2011.

[25] L. Suarez-Arrones, J. Tous-Fajardo, J. Nunez, O. Gonzalo-Skok, J. Galvez, and A. Mendez-Villanueva, "Concurrent repeated-sprint and resistance training with superimposed vibrations in rugby players," *Int J Sports Physiol Perform*, 2013.

[26] M. C. Marques, A. Pereira, I. G. Reis, and R. van den Tillaar, "Does an in-season 6-week combined sprint and jump training program improve strength-speed abilities and kicking performance in young soccer players?" *J Hum Kinet*, vol. 39, pp. 157–66, 2013.

[27] C. Kotzamanidis, D. Chatzopoulos, C. Michailidis, G. Papaiakovou, and D. Patikas, "The effect of a combined high-intensity strength and speed training program on the running and jumping ability of soccer players," *J Strength Cond Res*, vol. 19, no. 2, pp. 369–75, 2005.

[28] G. S. Cantrell, B. K. Schilling, M. R. Paquette, and Z. Murlasits, "Maximal strength, power, and aerobic endurance adaptations to concurrent strength and sprint interval training," *Eur J Appl Physiol*, vol. 114, no. 4, pp. 763–71, 2014.

[29] R. E. Ross, N. A. Ratamess, J. R. Hoffman, A. D. Faigenbaum, J. Kang, and A. Chilakos, "The effects of treadmill sprint training and resistance training on maximal running velocity and power," *J Strength Cond Res*, vol. 23, no. 2, pp. 385–94, 2009.

[30] K. McGawley and P. I. Andersson, "The order of concurrent training does not affect soccer-related performance adaptations," *Int J Sports Med*, vol. 34, no. 11, pp. 983–90, 2013.

[31] C. P. Fischer, "Interleukin-6 in acute exercise and training: what is the biological relevance?" *Exerc Immunol Rev*, vol. 12, pp. 6–33, 2006.

[32] M. A. Febbraio and B. K. Pedersen, "Muscle-derived interleukin-6: mechanisms for activation and possible biological roles," *FASEB J*, vol. 16, no. 11, pp. 1335–47, 2002.

[33] B. K. Pedersen, "Muscular interleukin-6 and its role as an energy sensor," *Med Sci Sports Exerc*, vol. 44, no. 3, pp. 392–6, 2012.

[34] J. Peake, K. Nosaka, and K. Suzuki, "Characterization of inflammatory responses to eccentric exercise in humans," *Exerc Immunol Rev*, vol. 11, pp. 64–85, 2005.

[35] M. A. Febbraio, N. Hiscock, M. Sacchetti, C. P. Fischer, and B. K. Pedersen, "Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction," *Diabetes*, vol. 53, no. 7, pp. 1643–8, 2004.

[36] M. Febbraio *et al.*, "Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans," *J Physiol (Lond)*, vol. 549, no. 2, pp. 607–612, 2003.

[37] R. L. Starkie, M. J. Arkinstall, I. Koukoulas, J. A. Hawley, and M. A. Febbraio, "Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans," *J*

Physiol, vol. 533, no. 2, pp. 585–591, 2001.

[38] L. L. Smith, A. Anwar, M. Fragen, C. Rananto, R. Johnson, and D. Holbert, "Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise," *Eur J Appl Physiol*, vol. 82, no. 1-2, pp. 61–7, 2000.

[39] M. H. Chan, A. L. Carey, M. J. Watt, and M. A. Febbraio, "Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that il-8, like il-6, is influenced by glycogen availability," *Am J Physiol Regul Integr Comp Physiol*, vol. 287, no. 2, pp. R322–7, 2004.

[40] I. H. Jonsdottir, P. Schjerling, K. Ostrowski, S. Asp, E. A. Richter, and B. K. Pedersen, "Muscle contractions induce interleukin-6 mRNA production in rat skeletal muscles," *J Physiol*, vol. 528 Pt 1, pp. 157–63, 2000.

[41] D. Nieman *et al.*, "Muscle cytokine mRNA changes after 2.5 h of cycling: Influence of carbohydrate," *Med Sci Sports Exerc*, vol. 37, no. 8, pp. 1283–1290, 2005.

[42] D. Nieman *et al.*, "Influence of mode and carbohydrate on the cytokine response to heavy exertion." *Med Sci Sports Exerc*, vol. 30, no. 5, pp. 671–678, 1998.

[43] Y. Meckel, A. Eliakim, M. Seraev, F. Zaldivar, D. M. Cooper, M. Sagiv, and D. Nemet, "The effect of a brief sprint interval exercise on growth factors and inflammatory mediators," *J Strength Cond Res*, vol. 23, no. 1, pp. 225–30, 2009.

[44] Y. Meckel, D. Nemet, S. Bar-Sela, S. Radom-Aizik, D. M. Cooper, M. Sagiv, and A. Eliakim, "Hormonal and inflammatory responses to different types of sprint interval training," *J Strength Cond Res*, vol. 25, no. 8, pp. 2161–9, 2011.

[45] R. K. Dishman, "Prescribing exercise intensity for healthy adults using perceived exertion," *Med Sci Sports Exerc*, vol. 26, no. 9, pp. 1087–94, 1994.

[46] J. P. McCarthy, M. A. Pozniak, and J. C. Agre, "Neuromuscular adaptations to concurrent strength and endurance training," *Med Sci Sports Exerc*, vol. 34, no. 3, pp. 511–9, 2002.

[47] C. P. Balabinis, C. H. Psarakis, M. Moukas, M. P. Vassiliou, and P. K. Behrakis, "Early phase changes by concurrent endurance and strength training," *J Strength Cond Res*, vol. 17, no. 2, pp. 393–401, 2003.

[48] G. T. Levin, M. R. McGuigan, and P. B. Laursen, "Effect of concurrent resistance and endurance training on physiologic and performance parameters of well-trained endurance cyclists," *J Strength Cond Res*, vol. 23, no. 8, pp. 2280–6, 2009.

[49] C. Hausswirth, S. Argentin, F. Bieuzen, Y. Le Meur, A. Couturier, and J. Brisswalter, "Endurance and strength training effects on physiological and muscular parameters during prolonged cycling," *J Electromyogr Kinesiol*, vol. 20, no. 2, pp. 330–9, 2010.

[50] B. L. Gravelle and D. L. Blessing, "Physiological adaptation in women concurrently training for strength and endurance," *J Strength Cond Res*, vol. 14, no. 1, pp. 5–13, 2000.

[51] D. G. Sale, I. Jacobs, J. D. MacDougall, and S. Garner, "Comparison of two regimens of concurrent strength and endurance training," *Med Sci Sports Exerc*, vol. 22, no. 3, pp. 348–56, 1990.

[52] B. W. Craig, J. Lucas, R. Pohlman, and H. Stelling, "The effects of running, weightlifting and a combination of both on growth hormone release," *J Strength Cond Res*, vol. 5, no. 4, pp. 198–203, 1991.

[53] K. R. Howarth, S. M. Phillips, M. J. MacDonald, D. Richards, N. A. Moreau, and M. J. Gibala, "Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery," *J Appl Physiol*, vol. 109, no. 2, pp. 431–8, 2010.

[54] A. Creer, P. Gallagher, D. Slivka, B. Jemiolo, W. Fink, and S. Trappe, "Influence of muscle glycogen availability on ERK1/2 and Akt signaling after resistance exercise in human skeletal muscle," *J Appl Physiol*, vol. 99, no. 3, pp. 950–6, 2005.

[55] E. G. Churchley, V. G. Coffey, D. J. Pedersen, A. Shield, K. A. Carey, D. Cameron-Smith, and J. A. Hawley, "Influence of preexercise muscle glycogen content on transcriptional activity of metabolic and myogenic genes in well-trained humans," *J Appl Physiol (1985)*, vol. 102, no. 4, pp. 1604–11, 2007.

[56] D. M. Camera, D. W. West, N. A. Burd, S. M. Phillips, A. P. Garnham, J. A. Hawley, and V. G. Coffey, "Low muscle glycogen concentration does not suppress the anabolic response to resistance exercise," *J Appl Physiol (1985)*, vol. 113, no. 2, pp. 206–14, 2012.

[57] B. Egan and J. Zierath, "Exercise metabolism and the molecular regulation of skeletal muscle adaptation," *Cell Metabolism*, vol. 17, no. 2, pp. 162 – 184, 2013.

[58] J. A. Hawley, "Molecular responses to strength and endurance training: are they incompatible?" *Appl Physiol Nutr Metab*, vol. 34, no. 3, pp. 355–61, 2009.

[59] V. G. Coffey and J. A. Hawley, "The molecular bases of training adaptation," *Sports Med*, vol. 37, no. 9, pp. 737–63, 2007.

[60] K. Baar, "Training for endurance and strength: lessons from cell signaling," *Med Sci Sports Exerc*, vol. 38, no. 11, pp. 1939–44, 2006.

[61] K. Inoki, T. Zhu, and K. L. Guan, "TSC2 mediates cellular energy response to control cell growth and survival," *Cell*, vol. 115, no. 5, pp. 577–90, 2003.

[62] D. M. Gwinn *et al.*, "AMPK phosphorylation of raptor mediates a metabolic checkpoint," *Mol Cell*, vol. 30, no. 2, pp. 214–26, 2008.

[63] K. Nakashima and Y. Yakabe, "Ampk activation stimulates myofibrillar protein degradation and expression of atrophy-related ubiquitin ligases by increasing foxo transcription factors in c2c12 myotubes," *Biosci Biotechnol Biochem*, vol. 71, no. 7, pp. 1650–6, 2007.

[64] A. M. Sanchez, A. Csibi, A. Raibon, K. Cornille, S. Gay, H. Bernardi, and R. Candau, "Ampk promotes skeletal muscle autophagy through activation of forkhead foxo3a and interaction with ulk1," *J Cell Biochem*, vol. 113, no. 2, pp. 695–710, 2012.

[65] T. N. Stitt *et al.*, "The igf-1/pi3k/akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting foxo transcription factors," *Mol Cell*, vol. 14, no. 3, pp. 395–403, 2004.

[66] M. Sandri *et al.*, "Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy," *Cell*, vol. 117, no. 3, pp. 399–412, 2004.

[67] H. Mascher, B. Ekblom, O. Rooyackers, and E. Blomstrand, "Enhanced rates of muscle protein synthesis and elevated mTOR signalling following endurance exercise in human subjects," *Acta Physiol (Oxf)*, vol. 202, no. 2, pp. 175–84, 2011.

[68] G. D. Wadley, R. J. Tunstall, A. Sanigorski, G. R. Collier, M. Hargreaves, and D. Cameron-Smith, "Differential effects of exercise on insulin-signaling gene expression in human skeletal muscle," *J Appl Physiol*, vol. 90, no. 2, pp. 436–440, 2001.

[69] S. B. Wilkinson, S. M. Phillips, P. J. Atherton, R. Patel, K. E. Yarasheski, M. A. Tarnopolsky, and M. J. Rennie, "Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle," *J Physiol*, vol. 586, no. Pt 15, pp. 3701–17, 2008.

[70] C. E. Donges *et al.*, "Concurrent resistance and aerobic exercise stimulates both myofibrillar and mitochondrial protein synthesis in sedentary middle-aged men," *J Appl Physiol*, vol. 112, no. 12, pp. 1992–2001, 2012.

[71] V. G. Coffey, H. Pilegaard, A. P. Garnham, B. J. O'Brien, and J. A. Hawley, "Consecutive bouts of diverse contractile activity alter acute responses in human skeletal muscle," *J Appl Physiol*, vol. 106, no. 4, pp. 1187–1197, 2009.

[72] V. G. Coffey, B. Jemiolo, J. Edge, A. P. Garnham, S. W. Trappe, and J. A. Hawley, "Effect of consecutive repeated sprint and resistance exercise bouts on acute adaptive responses in human skeletal muscle," *Am J Physiol Regul Integr Comp Physiol*, vol. 297, no. 5, pp. R1441–51, 2009.

[73] P. J. Atherton, J. Babraj, K. Smith, J. Singh, M. J. Rennie, and H. Wackerhage, "Selective activation of AMPK-PGC-1alpha or PKB-TSC2-mTOR signaling can explain specific adaptive responses to endurance or resistance training-like electrical muscle stimulation," *FASEB J*, vol. 19, no. 7, pp. 786–8, 2005.

[74] L. Wang, H. Mascher, N. Psilander, E. Blomstrand, and K. Sahlin, "Resistance exercise enhances the molecular signaling of mitochondrial biogenesis induced by endurance exercise in human skeletal muscle," *J Appl Physiol*, vol. 111, no. 5, pp. 1335–44, 2011.

[75] C. J. Mitchell *et al.*, "Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men," *PLoS One*, vol. 9, no. 2, p. e89431, 2014.

[76] C. J. Mitchell, T. A. Churchward-Venne, D. W. D. West, N. A. Burd, L. Breen, S. K. Baker, and S. M. Phillips, "Resistance exercise load does not determine training-mediated hypertrophic gains in young men," *J Appl Physiol*, vol. 113, no. 1, pp. 71–77, 2012.

[77] E. O. de Souza *et al.*, "Molecular adaptations to concurrent training," *Int J Sports Med*, vol. 34, no. 3, pp. 207–13, 2013.

[78] J. J. Fyfe, D. J. Bishop, and N. K. Stepto, "Interference between concurrent resistance and endurance exercise: molecular bases and the role of individual training variables," *Sports Med*, vol. 44, no. 6, pp. 743–62, 2014.

[79] M. C. Schaub, U. T. Brunner, C. Von Schulthess, M. Neidhart, and H. Baumann, "Adaptation of contractile proteins in human heart and skeletal muscles," *Biomed Biochim Acta*, vol. 48, no. 5-6, pp. S306–12, 1989.

[80] S. Trappe, M. Harber, A. Creer, P. Gallagher, D. Slivka, K. Minchev, and D. Whitsett, "Single muscle fiber adaptations with marathon training," *J Appl Physiol*, vol. 101, no. 3, pp. 721–727, 2006.

[81] J. J. Widrick, J. E. Stelzer, T. C. Shoepe, and D. P. Garner, "Functional properties of human muscle fibers after short-term resistance exercise training," *Am J Physiol Regul Integr Comp Physiol*, vol. 283, no. 2, pp. R408–16, 2002.

[82] L. Malisoux, M. Francaux, and D. Theisen, "What do single-fiber studies tell us about exercise training?" *Med Sci Sports Exerc*, vol. 39, no. 7, pp. 1051–60, 2007.

[83] A. G. Nelson, D. A. Arnall, S. F. Loy, L. J. Silvester, and R. K. Conlee, "Consequences of combining strength and endurance training regimens," *Phys Ther*, vol. 70, no. 5, pp. 287–94, 1990.

[84] J. Duchateau, J. G. Semmler, and R. M. Enoka, "Training adaptations in the behavior of human motor units," *J Appl Physiol*, vol. 101, no. 6, pp. 1766–1775, 2006.

[85] T. Moritani and H. A. deVries, "Neural factors versus hypertrophy in the time course of muscle strength gain," *Am J Phys Med*, vol. 58, no. 3, pp. 115–30, 1979.

[86] J. P. Folland and A. G. Williams, "The adaptations to strength training : morphological and neurological contributions to increased strength," *Sports Med*, vol. 37, no. 2, pp. 145–68, 2007.

[87] N. A. Tillin, M. T. Pain, and J. P. Folland, "Short-term unilateral resistance training affects the agonist-antagonist but not the force-agonist activation relationship," *Muscle Nerve*, vol. 43, no. 3, pp. 375–84, 2011.

[88] C. Del Balso and E. Cafarelli, "Adaptations in the activation of human skeletal muscle induced by short-term isometric resistance training," *J Appl Physiol*, vol. 103, no. 1, pp. 402–11, 2007.

[89] B. K. Barry, G. E. Warman, and R. G. Carson, "Age-related differences in rapid muscle activation after rate of force development training of the elbow flexors," *Exp Brain Res*, vol. 162, no. 1, pp. 122–32, 2005.

[90] N. A. Tillin, M. T. Pain, and J. P. Folland, "Short-term training for explosive strength causes neural and mechanical adaptations," *Exp Physiol*, vol. 97, no. 5, pp. 630–41, 2012.

[91] K. Häkkinen, A. Pakarinen, H. Kyrolainen, S. Cheng, D. H. Kim, and P. V.

Komi, "Neuromuscular adaptations and serum hormones in females during prolonged power training," *Int J Sports Med*, vol. 11, no. 2, pp. 91–8, 1990.

[92] N. A. Tillin and J. P. Folland, "Maximal and explosive strength training elicit distinct neuromuscular adaptations, specific to the training stimulus," *Eur J Appl Physiol*, vol. 114, no. 2, pp. 365–74, 2014.

[93] J. Mikkola, H. Rusko, A. Nummela, T. Pollari, and K. Hakkinen, "Concurrent endurance and explosive type strength training improves neuromuscular and anaerobic characteristics in young distance runners," *Int J Sports Med*, vol. 28, no. 7, pp. 602–11, 2007.

[94] B. R. Ronnestad, E. A. Hansen, and T. Raastad, "High volume of endurance training impairs adaptations to 12 weeks of strength training in well-trained endurance athletes," *Eur J Appl Physiol*, vol. 112, no. 4, pp. 1457–66, 2012.

[95] E. L. Cadore *et al.*, "Neuromuscular adaptations to concurrent training in the elderly: effects of intrasession exercise sequence," *Age*, vol. 35, no. 3, pp. 891–903, 2013.

[96] M. Chtara *et al.*, "Effects of intra-session concurrent endurance and strength training sequence on aerobic performance and capacity," *Br J Sports Med*, vol. 39, no. 8, pp. 555–60, 2005.

[97] M. Chtara, A. Chaouachi, G. T. Levin, M. Chaouachi, K. Chamari, M. Amri, and P. B. Laursen, "Effect of concurrent endurance and circuit resistance training sequence on muscular strength and power development," *J Strength Cond Res*, vol. 22, no. 4, pp. 1037–45, 2008.

[98] E. L. Cadore *et al.*, "Hormonal responses to concurrent strength and endurance training with different exercise orders," *J Strength Cond Res*, vol. 26, no. 12, pp. 3281–8, 2012.

[99] R. S. Taipale and K. Hakkinen, "Acute hormonal and force responses to combined strength and endurance loadings in men and women: the "order effect"," *PLoS One*, vol. 8, no. 2, p. e55051, 2013.

[100] E. E. Hill, E. Zack, C. Battaglini, M. Viru, A. Viru, and A. C. Hackney, "Exercise and circulating cortisol levels: the intensity threshold effect," *J Endocrinol Invest*, vol. 31, no. 7, pp. 587–91, 2008.

[101] M. R. McGuigan, A. D. Egan, and C. Foster, "Salivary cortisol responses and perceived exertion during high intensity and low intensity bouts of resistance exercise," *J Sports Sci Med*, vol. 3, no. 1, pp. 8–15, 2004.

[102] V. Linnamo, A. Pakarinen, P. V. Komi, W. J. Kraemer, and K. Hakkinen, "Acute hormonal responses to submaximal and maximal heavy resistance and explosive exercises in men and women," *J Strength Cond Res*, vol. 19, no. 3, pp. 566–71, 2005.

[103] M. Schumann, S. Walker, M. Izquierdo, R. U. Newton, W. J. Kraemer, and K. Hakkinen, "The order effect of combined endurance and strength loadings on force and hormone responses: effects of prolonged training," *Eur J Appl Physiol*, vol. 114, no. 4, pp. 867–80, 2014.

[104] D. W. D. West *et al.*, "Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor

strength of the elbow flexors," *J Appl Physiol*, vol. 108, no. 1, pp. 60–67, 2010.

[105] D. D. West and S. Phillips, "Associations of exercise-induced hormone profiles and gains in strength and hypertrophy in a large cohort after weight training," *Eur J Appl Physiol*, vol. 112, no. 7, pp. 2693–2702, 2012.

[106] E. T. Schroeder, M. Villanueva, D. D. West, and S. M. Phillips, "Are acute post-resistance exercise increases in testosterone, growth hormone, and igf-1 necessary to stimulate skeletal muscle anabolism and hypertrophy?" *Med Sci Sports Exerc*, vol. 45, no. 11, pp. 2044–51, 2013.

[107] B. J. Schoenfeld, "Postexercise hypertrophic adaptations: a reexamination of the hormone hypothesis and its applicability to resistance training program design," *J Strength Cond Res*, vol. 27, no. 6, pp. 1720–30, 2013.

[108] D. W. West and S. M. Phillips, "Anabolic processes in human skeletal muscle: restoring the identities of growth hormone and testosterone," *Phys Sportsmed*, vol. 38, no. 3, pp. 97–104, 2010.

[109] V. Di Salvo, R. Baron, H. Tschan, F. J. Calderon Montero, N. Bachl, and F. Pigozzi, "Performance characteristics according to playing position in elite soccer," *Int J Sports Med*, vol. 28, no. 3, pp. 222–7, 2007.

[110] P. Krustrup, M. Mohr, H. Ellingsgaard, and J. Bangsbo, "Physical demands during an elite female soccer game: importance of training status," *Med Sci Sports Exerc*, vol. 37, no. 7, pp. 1242–1248, 2005.

[111] T. J. Gabbett, "Science of rugby league football: a review," *J Sports Sci*, vol. 23, no. 9, pp. 961–76, 2005.

[112] J. Bloomfield, R. Polman, and P. O'Donoghue, "Physical demands of different positions in fa premier league soccer," *J Sports Sci Med*, vol. 6, no. 1, pp. 63–70, 2007.

[113] A. Coutts, P. Reaburn, and G. Abt, "Heart rate, blood lactate concentration and estimated energy expenditure in a semi-professional rugby league team during a match: a case study," *J Sports Sci*, vol. 21, no. 2, pp. 97–103, 2003.

[114] D. A. McLean, "Analysis of the physical demands of international rugby union," *J Sports Sci*, vol. 10, no. 3, pp. 285–96, 1992.

[115] P. Krustrup, M. Mohr, A. Steensberg, J. Bencke, M. Kjaer, and J. Bangsbo, "Muscle and blood metabolites during a soccer game: implications for sprint performance," *Med Sci Sports Exerc*, vol. 38, no. 6, pp. 1165–74, 2006.

[116] J. D. Vescovi, "Sprint profile of professional female soccer players during competitive matches: Female Athletes in Motion (FAiM) study," *J Sports Sci*, vol. 30, no. 12, pp. 1259–65, 2012.

[117] M. Andrzejewski, J. Chmura, B. Pluta, R. Strzelczyk, and A. Kasprzak, "Analysis of sprinting activities of professional soccer players," *J Strength Cond Res*, vol. 27, no. 8, pp. 2134–40, 2013.

[118] M. Spencer, D. Bishop, B. Dawson, and C. Goodman, "Physiological and metabolic responses of repeated-sprint activities:specific to field-based team sports," *Sports Med*, vol. 35, no. 12, pp. 1025–44, 2005.

[119] C. Castagna, F. M. Impellizzeri, K. Chamari, D. Carlomagno, and E. Rampinini, "Aerobic fitness and yo-yo continuous and intermittent tests performances in soccer players: a correlation study," *J Strength Cond Res*, vol. 20, no. 2, pp. 320–5, 2006.

[120] O. Girard, A. Mendez-Villanueva, and D. Bishop, "Repeated-sprint ability - part i: factors contributing to fatigue," *Sports Med*, vol. 41, no. 8, pp. 673–94, 2011.

[121] T. Reilly, J. Bangsbo, and A. Franks, "Anthropometric and physiological predispositions for elite soccer," *J Sports Sci*, vol. 18, no. 9, pp. 669–83, 2000.

[122] J. B. Cronin and K. T. Hansen, "Strength and power predictors of sports speed," *J Strength Cond Res*, vol. 19, no. 2, pp. 349–57, 2005.

[123] A. Chaouachi, M. Brughelli, K. Chamari, G. T. Levin, N. Ben Abdelkrim, L. Laurencelle, and C. Castagna, "Lower limb maximal dynamic strength and agility determinants in elite basketball players," *J Strength Cond Res*, vol. 23, no. 5, pp. 1570–7, 2009.

[124] D. S. Lorenz, M. P. Reiman, B. Lehecka, and A. Naylor, "What performance characteristics determine elite versus nonelite athletes in the same sport?" *Sports Health*, vol. 5, no. 6, pp. 542–547, 2013.

[125] J. M. Wilson, P. J. Marin, M. R. Rhea, S. M. Wilson, J. P. Loenneke, and J. C. Anderson, "Concurrent training: a meta-analysis examining interference of aerobic and resistance exercises," *J Strength Cond Res*, vol. 26, no. 8, pp. 2293–307, 2012.

[126] S. A. Shalfawi, T. Haugen, T. A. Jakobsen, E. Enoksen, and E. Tonnessen, "The effect of combined resisted agility and repeated sprint training vs. strength training on female elite soccer players," *J Strength Cond Res*, vol. 27, no. 11, pp. 2966–72, 2013.

[127] D. Bishop, O. Girard, and A. Mendez-Villanueva, "Repeated-sprint ability - part II: recommendations for training," *Sports Med*, vol. 41, no. 9, pp. 741–56, 2011.

[128] K. A. Burgomaster, S. C. Hughes, G. J. F. Heigenhauser, S. N. Bradwell, and M. J. Gibala, "Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans," *J Appl Physiol*, vol. 98, no. 6, pp. 1985–1990, 2005.

[129] M. J. Gibala *et al.*, "Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance," *J Physiol*, vol. 575, no. 3, pp. 901–911, 2006.

[130] M. Esbjörnsson-Liljedahl, C. J. Sundberg, B. Norman, and E. Jansson, "Metabolic response in type I and type II muscle fibers during a 30-s cycle sprint in men and women," *J Appl Physiol*, vol. 87, no. 4, pp. 1326–1332, 1999.

[131] W. J. Kraemer *et al.*, "Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females," *Int J Sports Med*, vol. 12, no. 2, pp. 228–35, 1991.

[132] G. A. Thomas, W. J. Kramer, B. A. Spiering, J. S. Volek, J. Anderson, and

C. Maresh, "Maximal power at different percentages of one repetition maximum: Influence of resistance and gender," *J Strength Cond Res*, vol. 21, no. 2, pp. 336–342, 2007.

[133] K. Ostrowski, T. Rohde, S. Asp, P. Schjerling, and B. K. Pedersen, "Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans," *J Physiol*, vol. 515 (Pt 1), pp. 287–91, 1999.

[134] K. Suzuki, S. Nakaji, M. Yamada, M. Totsuka, K. Sato, and K. Sugawara, "Systemic inflammatory response to exhaustive exercise. cytokine kinetics," *Exerc Immunol Rev*, vol. 8, pp. 6–48, 2002.

[135] B. K. Pedersen, "Special feature for the olympics: effects of exercise on the immune system: exercise and cytokines," *Immunol Cell Biol*, vol. 78, no. 5, pp. 532–5, 2000.

[136] A. Steensberg, G. van Hall, T. Osada, M. Sacchetti, B. Saltin, and B. K. Pedersen, "Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6," *J Physiol*, vol. 529, no. 1, pp. 237–242, 2000.

[137] K. Ostrowski, C. Hermann, A. Bangash, P. Schjerling, J. N. Nielsen, and B. K. Pedersen, "A trauma-like elevation of plasma cytokines in humans in response to treadmill running," *J Physiol*, vol. 513, no. 3, pp. 889–894, 1998.

[138] D. C. Nieman, C. L. Dumke, D. A. Henson, S. R. McAnulty, S. J. Gross, and R. H. Lind, "Muscle damage is linked to cytokine changes following a 160-km race," *Brain Behav Immun*, vol. 19, no. 5, pp. 398–403, 2005.

[139] L. Croft *et al.*, "High-intensity interval training attenuates the exercise-induced increase in plasma il-6 in response to acute exercise," *Appl Physiol Nutr Metab*, vol. 34, no. 6, pp. 1098–107, 2009.

[140] C. Lundby and A. Steensberg, "Interleukin-6 response to exercise during acute and chronic hypoxia," *Eur J Appl Physiol*, vol. 91, no. 1, pp. 88–93, 2004.

[141] A. M. Niess, E. Fehrenbach, R. Lehmann, L. Opavsky, M. Jesse, H. Northoff, and H. H. Dickhuth, "Impact of elevated ambient temperatures on the acute immune response to intensive endurance exercise," *Eur J Appl Physiol*, vol. 89, no. 3-4, pp. 344–51, 2003.

[142] C. MacDonald, J. F. Wojtaszewski, B. K. Pedersen, B. Kiens, and E. A. Richter, "Interleukin-6 release from human skeletal muscle during exercise: relation to AMPK activity," *J Appl Physiol*, vol. 95, no. 6, pp. 2273–7, 2003.

[143] H. Bruunsgaard, H. Galbo, J. Halkjaer-Kristensen, T. L. Johansen, D. A. MacLean, and B. K. Pedersen, "Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage," *J Physiol*, vol. 499 (Pt 3), pp. 833–41, 1997.

[144] R. L. Starkie, M. Hargreaves, J. Rolland, and M. A. Febbraio, "Heat stress, cytokines, and the immune response to exercise," *Brain Behav Immun*, vol. 19, no. 5, pp. 404–12, 2005.

[145] H. Andersson, S. K. Bohn, T. Raastad, G. Paulsen, R. Blomhoff, and F. Kadi, "Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery," *Scand J Med Sci Sports*,

vol. 20, no. 5, pp. 740–7, 2010.

[146] C. Keller, A. Steensberg, H. Pilegaard, T. Osada, B. Saltin, B. K. Pedersen, and P. D. Neufer, "Transcriptional activation of the il-6 gene in human contracting skeletal muscle: influence of muscle glycogen content," *FASEB J*, vol. 15, no. 14, pp. 2748–50, 2001.

[147] C. P. Fischer, P. Plomgaard, A. K. Hansen, H. Pilegaard, B. Saltin, and B. K. Pedersen, "Endurance training reduces the contraction-induced interleukin-6 mRNA expression in human skeletal muscle," *Am J Physiol Endocrinol Metab*, vol. 287, no. 6, pp. E1189–94, 2004.

[148] J. W. Helge, B. Stallknecht, B. K. Pedersen, H. Galbo, B. Kiens, and E. A. Richter, "The effect of graded exercise on IL-6 release and glucose uptake in human skeletal muscle," *J Physiol*, vol. 546, no. Pt 1, pp. 299–305, 2003.

[149] M. De Rossi, P. Bernasconi, F. Baggi, R. de Waal Malefyt, and R. Mantegazza, "Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation." *Int Immunol*, vol. 12, no. 9, pp. 1329–1335, 2000.

[150] J. M. Peterson and F. X. Pizza, "Cytokines derived from cultured skeletal muscle cells after mechanical strain promote neutrophil chemotaxis in vitro," *J Appl Physiol*, vol. 106, no. 1, pp. 130–137, 2009.

[151] H. Ullum, P. M. Haahr, M. Diamant, J. Palmo, J. Halkjaer-Kristensen, and B. K. Pedersen, "Bicycle exercise enhances plasma IL-6 but does not change IL-1 alpha, IL-1 beta, IL-6, or TNF-alpha pre-mRNA in BMNC," *J Appl Physiol*, vol. 77, no. 1, pp. 93–7, 1994.

[152] R. L. Starkie, J. Rolland, D. J. Angus, M. J. Anderson, and M. A. Febbraio, "Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running," *Am J Physiol Cell Physiol*, vol. 280, no. 4, pp. C769–74, 2001.

[153] R. L. Starkie, D. J. Angus, J. Rolland, M. Hargreaves, and M. A. Febbraio, "Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans," *J Physiol*, vol. 528, no. Pt 3, pp. 647–55, 2000.

[154] P. Plomgaard, M. Penkowa, and B. K. Pedersen, "Fiber type specific expression of TNF-alpha, IL-6 and IL-18 in human skeletal muscles," *Exerc Immunol Rev*, vol. 11, pp. 53–63, 2005.

[155] P. A. Della Gatta, A. P. Garnham, J. M. Peake, and D. Cameron-Smith, "Effect of exercise training on skeletal muscle cytokine expression in the elderly," *Brain Behav Immun*, 2014.

[156] M. K. Trenerry, P. A. Della Gatta, A. E. Larsen, A. P. Garnham, and D. Cameron-Smith, "Impact of resistance exercise training on interleukin-6 and JAK/STAT in young men," *Muscle Nerve*, vol. 43, no. 3, pp. 385–92, 2011.

[157] J. L. Croisier *et al.*, "Effects of training on exercise-induced muscle damage and interleukin 6 production," *Muscle Nerve*, vol. 22, no. 2, pp. 208–12, 1999.

[158] C. Keller, Y. Hellsten, A. Steensberg, and B. K. Pedersen, "Differential regu-

lation of IL-6 and TNF-alpha via calcineurin in human skeletal muscle cells," *Cytokine*, vol. 36, no. 3-4, pp. 141–7, 2006.

[159] A. M. Petersen and B. K. Pedersen, "The anti-inflammatory effect of exercise," *J Appl Physiol*, vol. 98, no. 4, pp. 1154–62, 2005.

[160] S. L. Nehlsen-Cannarella *et al.*, "Carbohydrate and the cytokine response to 2.5 h of running," *J Appl Physiol*, vol. 82, no. 5, pp. 1662–1667, 1997.

[161] E. Kerasioti *et al.*, "Anti-inflammatory effects of a special carbohydrate-whey protein cake after exhaustive cycling in humans," *Food Chem Toxicol*, vol. 61, pp. 42–46, 2013.

[162] M. Miles, E. Walker, S. Conant, S. Hogan, and J. Kidd, "Carbohydrate influences plasma interleukin-6 but not c-reactive protein or creatine kinase following a 32-km mountain trail race," *Int J Sport Nutr Exerc Metab*, vol. 16, no. 1, pp. 36–46, 2006.

[163] N. Bishop, M. Gleeson, C. Nicholas, and A. Ali, "Influence of carbohydrate supplementation on plasma cytokine and neutrophil degranulation responses to high intensity intermittent exercise," *Int J Sport Nutr Exerc Metab*, vol. 12, no. 2, pp. 145–156, 2002.

[164] M. Gleeson and N. C. Bishop, "Special feature for the olympics: effects of exercise on the immune system: modification of immune responses to exercise by carbohydrate, glutamine and anti-oxidant supplements," *Immunol Cell Biol*, vol. 78, no. 5, pp. 554–61, 2000.

[165] N. Hiscock, M. H. S. Chan, T. Bisucci, I. A. Darby, and M. A. Febbraio, "Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity," *FASEB J*, 2004.

[166] M. P. Miles, S. D. Pearson, J. M. Andring, J. R. Kidd, and S. L. Volpe, "Effect of carbohydrate intake during recovery from eccentric exercise on interleukin-6 and muscle-damage markers," *Int J Sport Nutr Exerc Metab*, vol. 17, no. 6, pp. 507–520, 2007.

[167] M. Sim *et al.*, "The effects of carbohydrate ingestion during endurance running on post-exercise inflammation and hepcidin levels," *Eur J Appl Physiol*, vol. 112, no. 5, pp. 1889–1898, 2012.

[168] R. Afroundeh, M. Siahkouhian, and A. Khalili, "The effect of post-exercise carbohydrate ingestion on inflammatory responses to short time, high-force eccentric exercise," *J Sports Med Phys Fitness*, vol. 50, no. 2, pp. 182–188, 2010.

[169] M. L. R. Ross, S. L. Halson, K. Suzuki, A. Garnham, J. A. Hawley, D. Cameron-Smith, and J. M. Peake, "Cytokine responses to carbohydrate ingestion during recovery from exercise-induced muscle injury," *J Interferon Cytokine Res*, vol. 30, no. 5, pp. 329–337, 2010.

[170] A. Steensberg *et al.*, "Acute interleukin-6 administration does not impair muscle glucose uptake or whole-body glucose disposal in healthy humans," *J Physiol*, vol. 548, no. 2, pp. 631–638, 2003.

[171] E. W. Petersen, A. L. Carey, M. Sacchetti, G. R. Steinberg, S. L. Macaulay, M. A. Febbraio, and B. K. Pedersen, "Acute IL-6 treatment increases fatty

acid turnover in elderly humans in vivo and in tissue culture in vitro," *Am J Physiol Endocrinol Metab*, vol. 288, no. 1, pp. E155–E162, 2005.

[172] H. M. O'Neill, R. Palanivel, D. C. Wright, T. MacDonald, J. S. Lally, J. D. Schertzer, and G. R. Steinberg, "Il-6 is not essential for exercise-induced increases in glucose uptake," *J Appl Physiol*, vol. 114, no. 9, pp. 1151–7, 2013.

[173] A. L. Carey *et al.*, "Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via amp-activated protein kinase," *Diabetes*, vol. 55, no. 10, pp. 2688–97, 2006.

[174] N. Fujii, N. Jessen, and L. J. Goodyear, "AMP-activated protein kinase and the regulation of glucose transport," *Am J Physiol Endocrinol Metab*, vol. 291, no. 5, pp. E867–E877, 2006.

[175] B. R. McKay, M. De Lisio, A. P. W. Johnston, C. E. O'Reilly, and S. Phillips, "Association of interleukin-6 signalling with the muscle stem cell response following muscle-lengthening contractions in humans," *PLoS One*, vol. 4(6), 2009.

[176] K. G. Toth, B. R. McKay, M. De Lisio, J. P. Little, and M. A. Tarnopolsky, "IL-6 induced STAT3 signalling is associated with the proliferation of human muscle satellite cells following acute muscle damage." *PLoS One*, vol. 6(3, 2011.

[177] A. L. Serrano, B. Baeza-Raja, E. Perdiguero, M. Jardi, and P. Munoz-Canoves, "Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy," *Cell Metab*, vol. 7, no. 1, pp. 33–44, 2008.

[178] M. K. Trenerry, K. A. Carey, A. C. Ward, and D. Cameron-Smith, "STAT3 signaling is activated in human skeletal muscle following acute resistance exercise," *J Appl Physiol*, vol. 102, no. 4, pp. 1483–1489, 2007.

[179] Y. Yang *et al.*, "STAT3 induces muscle stem cell differentiation by interaction with myoD," *Cytokine*, vol. 46, no. 1, pp. 137–41, 2009.

[180] M. C. Calle and M. L. Fernandez, "Effects of resistance training on the inflammatory response," *Nutr Res Pract*, vol. 4, no. 4, pp. 259–269, 2010.

[181] J.-m. Cavaillon, M. Adib-conquy, C. Fitting, C. Adrie, and D. Payen, "Cytokine cascade in sepsis," *Scand J Infect Dis*, vol. 35, no. 9, pp. 535–544, 2003.

[182] K. Ostrowski, T. Rohde, M. Zacho, S. Asp, and B. K. Pedersen, "Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running," *J Physiol*, vol. 508 (Pt 3), pp. 949–53, 1998.

[183] A. Steensberg, C. P. Fischer, C. Keller, K. Møller, and B. K. Pedersen, "IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans," *Am J Physiol Endocrinol Metab*, vol. 285, no. 2, pp. E433–E437, 2003.

[184] C. Bogdan, J. Paik, Y. Vodovotz, and C. Nathan, "Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-beta and interleukin-10." *J Biol Chem*, vol. 267, no. 32, pp. 23 301–8, 1992.

[185] G. I. Lancaster *et al.*, "Effects of acute exhaustive exercise and chronic exercise training on type 1 and type 2 t lymphocytes," *Exerc Immunol Rev*, vol. 10, pp. 91–106, 2004.

[186] C. A. Dinarello, "The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1," *N Engl J Med*, vol. 343, no. 10, pp. 732–734, 2000.

[187] R. Schindler, J. Mancilla, S. Endres, R. Ghorbani, S. Clark, and C. Dinarello, "Correlations and interactions in the production of interleukin-6 (IL- 6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF," *Blood*, vol. 75, no. 1, pp. 40–47, 1990.

[188] L. Hirose, K. Nosaka, M. Newton, A. Laveder, M. Kano, J. Peake, and K. Suzuki, "Changes in inflammatory mediators following eccentric exercise of the elbow flexors," *Exerc Immunol Rev*, vol. 10, pp. 75–90, 2004.

[189] I. P. Oswald, T. A. Wynn, A. Sher, and S. L. James, "Interleukin 10 inhibits macrophage microbicidal activity by blocking the endogenous production of tumor necrosis factor alpha required as a costimulatory factor for interferon gamma-induced activation," *Proc Natl Acad Sci USA*, vol. 89, no. 18, pp. 8676–80, 1992.

[190] M. A. Bijjiga E, "Interleukin 10 (il-10) regulatory cytokine and its clinical consequences," *J Clin Cell Immunol*, vol. S1, p. 007, 2013.

[191] M. Baggiolini, "Novel aspects of inflammation: interleukin-8 and related chemotactic cytokines," *Clin Investig*, vol. 71, no. 10, pp. 812–814, 1993.

[192] J. M. Schröder, "Chemoattractants as mediators of neutrophilic tissue recruitment," *Clin Dermatol*, vol. 18, no. 3, pp. 245–263, 2000.

[193] J. Heidemann *et al.*, "Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2," *J Biol Chem*, vol. 278, no. 10, pp. 8508–8515, 2003.

[194] J. A. Belperio, M. P. Keane, D. A. Arenberg, C. L. Addison, J. E. Ehlert, M. D. Burdick, and R. M. Strieter, "CXC chemokines in angiogenesis," *J Leukocyte Biol*, vol. 68, no. 1, pp. 1–8, 2000.

[195] K. Ostrowski, T. Rohde, S. Asp, P. Schjerling, and B. K. Pedersen, "Chemokines are elevated in plasma after strenuous exercise in humans," *Eur J Appl Physiol*, vol. 84, no. 3, pp. 244–5, 2001.

[196] D. C. Nieman *et al.*, "Cytokine changes after a marathon race," *J Appl Physiol*, vol. 91, no. 1, pp. 109–114, 2001.

[197] D. C. Nieman *et al.*, "Influence of vitamin c supplementation on oxidative and immune changes after an ultramarathon," *J Appl Physiol*, vol. 92, no. 5, pp. 1970–1977, 2002.

[198] D. A. Henson *et al.*, "Influence of carbohydrate on cytokine and phagocytic responses to 2 h of rowing," *Med Sci Sports Exerc*, vol. 32, no. 8, pp. 1384–9, 2000.

[199] P. Mucci, F. Durand, B. Lebel, J. Bousquet, and C. Prefaut, "Interleukins 1-beta, -8, and histamine increases in highly trained, exercising athletes," *Med Sci Sports Exerc*, vol. 32, no. 6, pp. 1094–100, 2000.

[200] K. A. Zwetsloot, C. S. John, M. M. Lawrence, R. A. Battista, and R. A. Shanely, "High-intensity interval training induces a modest systemic inflam-

matory response in active, young men," *J Inflamm Res*, vol. 7, pp. 9–17, 2014.

[201] D. C. Nieman *et al.*, "Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training," *J Appl Physiol*, vol. 96, no. 4, pp. 1292–8, 2004.

[202] E. Louis, U. Raue, Y. Yang, B. Jemiolo, and S. Trappe, "Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle," *J Appl Physiol*, vol. 103, no. 5, pp. 1744–1751, 2007.

[203] T. Akerstrom, A. Steensberg, P. Keller, C. Keller, M. Penkowa, and B. K. Pedersen, "Exercise induces interleukin-8 expression in human skeletal muscle," *J Physiol*, vol. 563, no. Pt 2, pp. 507–16, 2005.

[204] D. C. Nieman *et al.*, "Carbohydrate ingestion influences skeletal muscle cytokine mrna and plasma cytokine levels after a 3-h run," *J Appl Physiol*, vol. 94, no. 5, pp. 1917–1925, 2003.

[205] L. Frydelund-Larsen, M. Penkowa, T. Akerstrom, A. Zankari, S. Nielsen, and B. K. Pedersen, "Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle," *Exp Physiol*, vol. 92, no. 1, pp. 233–40, 2007.

[206] A. Beutler, B. Cerami, "Tumor necrosis, cachexia, shock, and inflammation: a common mediator." *Annu Rev Biochem*, vol. 57, pp. 505–18, 1988.

[207] C. A. Dinarello, "The biological properties of interleukin-1," *Eur Cytokine Network*, vol. 5, no. 6, pp. 517–531, 1994.

[208] K. Suzuki *et al.*, "Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans," *Eur J Appl Physiol*, vol. 81, no. 4, pp. 281–7, 2000.

[209] K. Suzuki *et al.*, "Changes in markers of muscle damage, inflammation and HSP70 after an ironman triathlon race," *Eur J Appl Physiol*, vol. 98, no. 6, pp. 525–34, 2006.

[210] W. de Jager, K. Bourcier, G. T. Rijkers, B. J. Prakken, and V. Seyfert-Margolis, "Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays," *BMC Immunol*, vol. 10, p. 52, 2009.

[211] R. A. Fielding, T. J. Manfredi, W. Ding, M. A. Fiatarone, W. J. Evans, and J. G. Cannon, "Acute phase response in exercise. iii. neutrophil and IL-1 beta accumulation in skeletal muscle," *Am J Physiol*, vol. 265, no. 1 Pt 2, pp. R166–72, 1993.

[212] S. E. Riechman, G. Balasekaran, S. M. Roth, and R. E. Ferrell, "Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses," *J Appl Physiol*, vol. 97, no. 6, pp. 2214–9, 2004.

[213] L. S. Quinn, B. G. Anderson, R. H. Drivdahl, B. Alvarez, and J. M. Argiles, "Overexpression of interleukin-15 induces skeletal muscle hypertrophy in vitro: implications for treatment of muscle wasting disorders," *Exp Cell Res*, vol. 280, no. 1, pp. 55–63, 2002.

[214] P. S. Furmanczyk and L. S. Quinn, "Interleukin-15 increases myosin accretion in human skeletal myogenic cultures," *Cell Biol Int*, vol. 27, no. 10, pp. 845–

851, 2003.

[215] A. R. Nielsen *et al.*, "Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition," *J Physiol*, vol. 584, no. Pt 1, pp. 305–12, 2007.

[216] F. Haugen *et al.*, "Il-7 is expressed and secreted by human skeletal muscle cells," *Am J Physiol Cell Physiol*, vol. 298, no. 4, pp. C807–16, 2010.

[217] M. Konrad, D. C. Nieman, D. A. Henson, K. M. Kennerly, F. Jin, and S. J. Wallner-Liebmann, "The acute effect of ingesting a quercetin-based supplement on exercise-induced inflammation and immune changes in runners," *Int J Sport Nutr Exerc Metab*, vol. 21, no. 4, pp. 338–346, 2011.

[218] A. J. Cox, D. B. Pyne, P. U. Saunders, R. Callister, and M. Gleeson, "Cytokine responses to treadmill running in healthy and illness-prone athletes," *Med Sci Sports Exerc*, vol. 39, no. 11, pp. 1918–1926, 2007.

[219] J. Ostapiuk-Karolczuk, A. Zembron-Lacny, M. Naczk, M. Gajewski, A. Kasperska, H. Dziewiecka, and K. Szyszka, "Cytokines and cellular inflammatory sequence in non-athletes after prolonged exercise," *J Sports Med Phys Fitness*, vol. 52, no. 5, pp. 563–568, 2012.

[220] Y. J. Chen, S. H. S. Wong, C. O. W. Chan, C. K. Wong, C. W. Lam, and P. M. F. Siu, "Effects of glycemic index meal and cho-electrolyte drink on cytokine response and run performance in endurance athletes," *J Sci Med Sport*, vol. 12, no. 6, pp. 697–703, 2009.

[221] V. Boghrabadi, S. M. Hejazi, A. H. Gonabadi, H. Sanian, and F. Aminian, "Effects of moderate-intensity exercise on serum proinflammatory cytokine levels in obese and non-obese men," *Life Sci J*, vol. 9, no. 3, pp. 2529–2532, 2012.

[222] J. Peake, K. Suzuki, M. Hordern, G. Wilson, K. Nosaka, and J. Coombes, "Plasma cytokine changes in relation to exercise intensity and muscle damage," *Eur J Appl Physiol*, vol. 95, no. 5-6, pp. 514–521, 2005.

[223] G. T. Espersen, A. ElbæK, E. Ernst, E. Toft, S. Kaalund, C. Jersild, and N. Grunnet, "Effect of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood," *APMIS*, vol. 98, no. 1-6, pp. 395–400, 1990.

[224] P. M. Haahr *et al.*, "Effect of physical exercise on in vitro production of interleukin 1, interleukin 6, tumour necrosis factor-alpha, interleukin 2 and interferon-gamma," *Int J Sports Med*, vol. 12, no. 2, pp. 223–227, 1991.

[225] J. Bush *et al.*, "Exercise and recovery responses of lymphokines to heavy resistance exercise," *J Strength Cond Res*, vol. 14, no. 3, pp. 344–349, 2000.

[226] K. K. Hoyer, H. Dooms, L. Barron, and A. K. Abbas, "Interleukin-2 in the development and control of inflammatory disease," *Immunol Rev*, vol. 226, pp. 19–28, 2008.

[227] M. J. Edwards, F. N. Miller, D. E. Sims, D. L. Abney, D. A. Schuschke, and T. S. Corey, "Interleukin 2 acutely induces platelet and neutrophil-endothelial adherence and macromolecular leakage," *Cancer Res*, vol. 52, no. 12, pp. 3425–31, 1992.

[228] K. Nelms, A. D. Keegan, J. Zamorano, J. Ryan, and W. E. Paul, "The il-4 receptor: signaling mechanisms and biologic functions." *Annu Rev Immunol*, vol. 17, pp. 701–738, 1999.

[229] V. Horsley, K. M. Jansen, S. T. Mills, and G. K. Pavlath, "IL-4 acts as a myoblast recruitment factor during mammalian muscle growth," *Cell*, vol. 113, no. 4, pp. 483–94, 2003.

[230] O. Prokopchuk, Y. Liu, L. Wang, K. Wirth, D. Schmidtbleicher, and J. Steinacker, "Skeletal muscle IL-4, IL-4Ralpha, IL-13 and IL-13Ralpha1 expression and response to strength training." *Exerc Immunol Rev*, vol. 13, pp. 67–75, 2007.

[231] A. Tremblay, J. A. Simoneau, and C. Bouchard, "Impact of exercise intensity on body fatness and skeletal muscle metabolism," *Metabolism*, vol. 43, no. 7, pp. 814–8, 1994.

[232] P. A. Tesch, "Skeletal muscle adaptations consequent to long-term heavy resistance exercise," *Med Sci Sports Exerc*, vol. 20, no. 5 Suppl, pp. S132–4, 1988.

[233] J. Henriksson, "Training induced adaptation of skeletal muscle and metabolism during submaximal exercise," *J Physiol*, vol. 270, no. 3, pp. 661–675, 1977.

[234] J. O. Holloszy, "Biochemical adaptations to exercise: aerobic metabolism," *Exerc Sport Sci Rev*, vol. 1, pp. 45–71, 1973.

[235] H. J. Green, R. Helyar, M. Ball-Burnett, N. Kowalchuk, S. Symon, and B. Farrance, "Metabolic adaptations to training precede changes in muscle mitochondrial capacity," *J Appl Physiol*, vol. 72, no. 2, pp. 484–91, 1992.

[236] O. Ronsen, K. Holm, H. Staff, P. K. Opstad, B. K. Pedersen, and R. Bahr, "No effect of seasonal variation in training load on immuno-endocrine responses to acute exhaustive exercise," *Scand J Med Sci Sports*, vol. 11, no. 3, pp. 141–8, 2001.

[237] L. K. Stewart *et al.*, "The influence of exercise training on inflammatory cytokines and C-reactive protein," *Med Sci Sports Exerc*, vol. 39, no. 10, pp. 1714–9, 2007.

[238] J. Mäestu, J. Jürimäe, P. Purge, R. Rämson, and T. Jürimäe, "Performance improvement is associated with higher postexercise responses in interleukin-6 and tumor necrosis factor concentrations." *J Sports Med Phys Fitness*, vol. 50, no. 4, pp. 524–529, 2010.

[239] N. Farhangimaleki, F. Zehsaz, and P. M. Tiidus, "The effect of tapering period on plasma pro-inflammatory cytokine levels and performance in elite male cyclists," *J Sports Sci Med*, vol. 8, no. 4, pp. 600–6, 2009.

[240] M. Izquierdo *et al.*, "Cytokine and hormone responses to resistance training," *Eur J Appl Physiol*, vol. 107, no. 4, pp. 397–409, 2009.

[241] C. Keller, A. Steensberg, A. K. Hansen, C. P. Fischer, P. Plomgaard, and B. K. Pedersen, "Effect of exercise, training, and glycogen availability on il-6 receptor expression in human skeletal muscle," *J Appl Physiol*, vol. 99, no. 6, pp. 2075–2079, 2005.

[242] S. R. Gray, A. Ratkevicius, H. Wackerhage, P. Coats, and M. A. Nimmo, "The effect of interleukin-6 and the interleukin-6 receptor on glucose transport in mouse skeletal muscle." *Exp Physiol*, vol. 94(8), pp. 899–905, 2009.

[243] K. M. Edwards, V. E. Burns, C. Ring, and D. Carroll, "Individual differences in the interleukin-6 response to maximal and submaximal exercise tasks," *J Sports Sci*, vol. 24, no. 8, pp. 855–62, 2006.

[244] A. Tofighi, A. A. Ravasi, and J. T. Azar, "Gender differences between elite men and women handball players in response to inflammatory indices following one session of moderate resistance exercise," *Iran J Health and Phys Act*, vol. 3, no. 1, 2012.

[245] J. W. Helge, D. K. Klein, T. M. Andersen, G. van Hall, J. Calbet, R. Boushel, and B. Saltin, "Interleukin-6 release is higher across arm than leg muscles during whole-body exercise," *Experimental Physiology*, vol. 96, no. 6, pp. 590–598, 2011.

[246] B. W. Timmons, M. J. Hamadeh, M. C. Devries, and M. A. Tarnopolsky, "Influence of gender, menstrual phase, and oral contraceptive use on immunological changes in response to prolonged cycling," *J Appl Physiol*, vol. 99, no. 3, pp. 979–985, 2005.

[247] M. E. Chaffin, K. E. Berg, J. R. Meendering, T. L. Llewellyn, J. A. French, and J. E. Davis, "Interleukin-6 and delayed onset muscle soreness do not vary during the menstrual cycle," *Res Q Exerc Sport*, vol. 82, no. 4, pp. 693–701, 2011.

[248] B. Wisbey, P. G. Montgomery, D. B. Pyne, and B. Rattray, "Quantifying movement demands of afl football using gps tracking," *J Sci Med Sport*, vol. 13, no. 5, pp. 531–536, 2010.

[249] D. Casamichana, J. Castellano, and C. Castagna, "Comparing the physical demands of friendly matches and small-sided games in semiprofessional soccer players," *J Strength Cond Res*, vol. 26, no. 3, pp. 837–843, 2012.

[250] C. Cummins, R. Orr, H. OÕConnor, and C. West, "Global positioning systems (gps) and microtechnology sensors in team sports: A systematic review," *Sports Med*, vol. 43, no. 10, pp. 1025–1042, 2013.

[251] K. Sato, S. L. Smith, and W. A. Sands, "Validation of an accelerometer for measuring sport performance," *J Strength Cond Res*, vol. 23, no. 1, pp. 341–347, 2009.

[252] C. D. Paton and W. G. Hopkins, "Tests of cycling performance," *Sports Med*, vol. 31, no. 7, pp. 489–96, 2001.

[253] M. Buchheit, "Monitoring training status with HR measures: do all roads lead to rome?" *Front Physiol*, vol. 5, p. 73, 2014.

[254] A. D. Egan, J. B. Winchester, C. Foster, and M. R. McGuigan, "Using session RPE to monitor different methods of resistance exercise," *J Sports Sci Med*, vol. 5, no. 2, pp. 289–95, 2006.

[255] M. L. Day, M. R. McGuigan, G. Brice, and C. Foster, "Monitoring exercise intensity during resistance training using the session rpe scale," *J Strength Cond Res*, vol. 18, no. 2, pp. 353–8, 2004.

[256] A. B. Stannard, J. P. Brandenburg, W. A. Pitney, and J. M. Lukaszuk, "Effects of wearing a cooling vest during the warm-up on 10-km run performance," *J Strength Cond Res*, vol. 25, no. 7, pp. 2018–24, 2011.

[257] A. Lucia, J. Hoyos, M. Perez, and J. L. Chicharro, "Heart rate and performance parameters in elite cyclists: a longitudinal study," *Med Sci Sports Exerc*, vol. 32, no. 10, pp. 1777–82, 2000.

[258] T. Little and A. G. Williams, "Measures of exercise intensity during soccer training drills with professional soccer players," *J Strength Cond Res*, vol. 21, no. 2, pp. 367–71, 2007.

[259] R. G. Lockie, A. J. Murphy, B. R. Scott, and X. A. Janse de Jonge, "Quantifying session ratings of perceived exertion for field-based speed training methods in team sport athletes," *J Strength Cond Res*, vol. 26, no. 10, pp. 2721–8, 2012.

[260] M. Buchheit, M. B. Simpson, H. Al Haddad, P. C. Bourdon, and A. Mendez-Villanueva, "Monitoring changes in physical performance with heart rate measures in young soccer players," *Eur J Appl Physiol*, vol. 112, no. 2, pp. 711–23, 2012.

[261] P. G. Montgomery, D. J. Green, N. Etxebarria, D. B. Pyne, P. U. Saunders, and C. L. Minahan, "Validation of heart rate monitor-based predictions of oxygen uptake and energy expenditure," *J Strength Cond Res*, vol. 23, no. 5, pp. 1489–95, 2009.

[262] P. Wong del, C. Carling, A. Chaouachi, A. Dellal, C. Castagna, K. Chamari, and D. G. Behm, "Estimation of oxygen uptake from heart rate and ratings of perceived exertion in young soccer players," *J Strength Cond Res*, vol. 25, no. 7, pp. 1983–8, 2011.

[263] K. M. Stagno, R. Thatcher, and K. A. van Someren, "A modified trimp to quantify the in-season training load of team sport players," *J Sports Sci*, vol. 25, no. 6, pp. 629–34, 2007.

[264] J. Borresen and M. I. Lambert, "The quantification of training load, the training response and the effect on performance," *Sports Med*, vol. 39, no. 9, pp. 779–95, 2009.

[265] E. A. Algroy, K. J. Hetlelid, S. Seiler, and J. I. Stray Pedersen, "Quantifying training intensity distribution in a group of norwegian professional soccer players," *Int J Sports Physiol Perform*, vol. 6, no. 1, pp. 70–81, 2011.

[266] G. Borg, P. HassmŐn, and M. LagerstrŽm, "Perceived exertion related to heart rate and blood lactate during arm and leg exercise," *Eur J Appl Physiol*, vol. 56, no. 6, pp. 679–685, 1987.

[267] C. Twist and J. Highton, "Monitoring fatigue and recovery in rugby league players," *Int J Sports Physiol Perform*, vol. 8, no. 5, pp. 467–74, 2013.

[268] J. H. Van der Meulen, H. Kuipers, and J. Drukker, "Relationship between exercise-induced muscle damage and enzyme release in rats," *J Appl Physiol*, vol. 71, no. 3, pp. 999–1004, 1991.

[269] M. Tremblay, J. Copeland, and W. Van Helder, "Influence of exercise duration on post-exercise steroid hormone responses in trained males," *Eur J Appl Physiol*, vol. 94, no. 5-6, pp. 505–513, 2005.

[270] C. M. Maresh *et al.*, "Effect of hydration state on testosterone and cortisol responses to training-intensity exercise in collegiate runners," *Int J Sports Med*, vol. 27, no. 10, pp. 765–70, 2006.

[271] A. C. Fry and C. A. Lohnes, "Acute testosterone and cortisol responses to high power resistance exercise," *Fiziol Cheloveka*, vol. 36, no. 4, pp. 102–6, 2010.

[272] I. Ispirlidis *et al.*, "Time-course of changes in inflammatory and performance responses following a soccer game," *Clin J Sport Med*, vol. 18, no. 5, pp. 423–31, 2008.

[273] J. Edge, D. Bishop, S. Hill-Haas, B. Dawson, and C. Goodman, "Comparison of muscle buffer capacity and repeated-sprint ability of untrained, endurance-trained and team-sport athletes," *Eur J Appl Physiol*, vol. 96, no. 3, pp. 225–234, 2006.

[274] K. A. Burgomaster, G. J. F. Heigenhauser, and M. J. Gibala, "Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance," *J Appl Physiol*, vol. 100, no. 6, pp. 2041–2047, 2006.

[275] F. R. Serpiello, M. J. McKenna, D. J. Bishop, R. J. Aughey, M. K. Caldow, D. Cameron-Smith, and N. K. Stepto, "Repeated sprints alter signaling related to mitochondrial biogenesis in humans," *Med Sci Sports Exerc*, vol. 44, no. 5, pp. 827–34, 2012.

[276] S. M. Phillips, K. D. Tipton, A. Aarsland, S. E. Wolf, and R. R. Wolfe, "Mixed muscle protein synthesis and breakdown after resistance exercise in humans," *Am J Physiol Endocrinol Metab*, vol. 273, no. 1, pp. E99–E107, 1997.

[277] J. M. McBride, D. Blow, T. J. Kirby, T. L. Haines, A. M. Dayne, and N. T. Triplett, "Relationship between maximal squat strength and five, ten, and forty yard sprint times," *J Strength Cond Res*, vol. 23, no. 6, pp. 1633–6, 2009.

[278] P. Wahl, C. Zinner, S. Achtzehn, W. Bloch, and J. Mester, "Effect of high- and low-intensity exercise and metabolic acidosis on levels of GH, IGF-I, IGFBP-3 and cortisol," *Growth Horm. IGF Res*, vol. 20, no. 5, pp. 380 – 385, 2010.

[279] A. Steensberg, M. A. Febbraio, T. Osada, P. Schjerling, G. van Hall, B. Saltin, and B. K. Pedersen, "Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content," *J Physiol*, vol. 537, no. 2, pp. 633–639, 2001.

[280] R. P. Bernardes and M. W. Radomski, "Growth hormone responses to continuous and intermittent exercise in females under oral contraceptive therapy," *Eur J Appl Physiol Occup Physiol*, vol. 79, no. 1, pp. 24–9, 1998.

[281] C. Sunderland, V. Tunaley, F. Horner, D. Harmer, and K. A. Stokes, "Menstrual cycle and oral contraceptivesÕ effects on growth hormone response to sprinting," *Appl Physiol Nutr Metab*, vol. 36, no. 4, pp. 495–502, 2011.

[282] G. Masterson, "The impact of menstrual phases on anaerobic power performance in collegiate women," *J Strength Cond Res*, vol. 13, no. 4, pp. 325–329, 1999.

[283] K. J. Elliott-Sale *et al.*, "Examining the role of oral contraceptive users as an experimental and/or control group in athletic performance studies," *Contraception*, vol. 88, no. 3, pp. 408–12, 2013.

[284] J. L. Vingren *et al.*, "Effect of resistance exercise on muscle steroidogenesis," *J Appl Physiol*, vol. 105, no. 6, pp. 1754–60, 2008.

[285] P. Krustrup *et al.*, "The yo-yo intermittent recovery test: physiological response, reliability, and validity," *Med Sci Sports Exerc*, vol. 35, no. 4, pp. 697–705, 2003.

[286] J. Bangsbo, F. M. Iaia, and P. Krustrup, "The Yo-Yo intermittent recovery test : a useful tool for evaluation of physical performance in intermittent sports," *Sports Med*, vol. 38, no. 1, pp. 37–51, 2008.

[287] G. Borg, "Perceived exertion as an indicator of somatic stress," *Scand J Rehabil Med*, vol. 2, no. 2, pp. 92–8, 1970.

[288] G. Mastorakos, G. P. Chrousos, and J. S. Weber, "Recombinant interleukin-6 activates the hypothalamic-pituitary-adrenal axis in humans," *J Clin Endocrinol Metab*, vol. 77, no. 6, pp. 1690–4, 1993.

[289] C. Tsigos, D. A. Papanicolaou, R. Defensor, C. S. Mitsiadis, I. Kyrou, and G. P. Chrousos, "Dose effects of recombinant human lnterleukin-6 on pituitary hormone secretion and energy expenditure," *Neuroendocrinology*, vol. 66, no. 1, pp. 54–62, 1997.

[290] M. A. Minetto, A. Rainoldi, M. Gazzoni, G. P. Ganzit, L. Saba, and P. Paccotti, "Interleukin-6 response to isokinetic exercise in elite athletes: relationships to adrenocortical function and to mechanical and myoelectric fatigue," *Eur J Appl Physiol*, vol. 98, no. 4, pp. 373–82, 2006.

[291] H. Wright, T. McLellan, J. Stapleton, S. Hardcastle, and G. Kenny, "Cortisol and interleukin-6 responses during intermittent exercise in two different hot environments with equivalent WBGT." *J Occup Environ Hyg*, vol. 9, no. 4, pp. 269–279, 2012.

[292] B. K. Pedersen *et al.*, "The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor?" *Proc Nutr Soc*, vol. 63, no. 2, pp. 263–7, 2004.

[293] A. Steensberg, C. Keller, R. L. Starkie, T. Osada, M. A. Febbraio, and B. K. Pedersen, "IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle," *Am J Physiol Endocrinol Metab*, vol. 283, no. 6, pp. E1272–8, 2002.

[294] W. J. Kraemer *et al.*, "Hormonal and growth factor responses to heavy resistance exercise protocols," *J Appl Physiol*, vol. 69, no. 4, pp. 1442–50, 1990.

[295] W. J. Kraemer *et al.*, "Changes in hormonal concentrations after different heavy-resistance exercise protocols in women," *J Appl Physiol*, vol. 75, no. 2, pp. 594–604, 1993.

[296] K. Stokes, M. Nevill, J. Frystyk, H. Lakomy, and G. Hall, "Human growth hormone responses to repeated bouts of sprint exercise with different recovery periods between bouts," *J Appl Physiol*, vol. 99, no. 4, pp. 1254–61, 2005.

[297] W. J. Kraemer *et al.*, "Effects of heavy-resistance training on hormonal re-

sponse patterns in younger vs. older men," *J Appl Physiol*, vol. 87, no. 3, pp. 982–92, 1999.

[298] E. L. Cadore *et al.*, "Hormonal responses to resistance exercise in long-term trained and untrained middle-aged men," *J Strength Cond Res*, vol. 22, no. 5, pp. 1617–24, 2008.

[299] P. Le Rossignol, T. J. Gabbett, D. Comerford, and W. R. Stanton, "Repeated sprint ability and team selection in australian football league players," *Int J Sports Physiol Perform*, vol. 23, p. 23, 2013.

[300] U. Wisløff, J. Helgerud, and J. Hoff, "Strength and endurance of elite soccer players," *Med Sci Sports Exerc*, vol. 30, no. 3, pp. 462–7, 1998.

[301] G. J. Bell, D. Syrotuik, T. P. Martin, R. Burnham, and H. A. Quinney, "Effect of concurrent strength and endurance training on skeletal muscle properties and hormone concentrations in humans," *Eur J Appl Physiol*, vol. 81, no. 5, pp. 418–27, 2000.

[302] S. P. Glowacki, S. E. Martin, A. Maurer, W. Baek, J. S. Green, and S. F. Crouse, "Effects of resistance, endurance, and concurrent exercise on training outcomes in men," *Med Sci Sports Exerc*, vol. 36, no. 12, pp. 2119–27, 2004.

[303] D. G. Sale, J. D. MacDougall, I. Jacobs, and S. Garner, "Interaction between concurrent strength and endurance training," *J Appl Physiol*, vol. 68, no. 1, pp. 260–70, 1990.

[304] M. Leveritt and P. J. Abernethy, "Acute effects of high-intensity endurance exercise on subsequent resistance activity," *J Strength Cond Res*, vol. 13, no. 1, pp. 47–51, 1999.

[305] G. Paulsen, U. R. Mikkelsen, T. Raastad, and J. M. Peake, "Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise?" *Exerc Immunol Rev*, vol. 18, pp. 42–97, 2012.

[306] M. Fitzsimmons, B. Dawson, D. Ward, and A. Wilkinson, "Cycling and running tests of repeated sprint ability." *Aust J Sci Med Sport*, vol. 25, no. 4, pp. 82 – 87, 1993.

[307] M. R. McGuigan, T. L. Doyle, M. Newton, D. J. Edwards, S. Nimphius, and R. U. Newton, "Eccentric utilization ratio: effect of sport and phase of training," *J Strength Cond Res*, vol. 20, no. 4, pp. 992–5, 2006.

[308] D. B. Dill and D. L. Costill, "Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration," *J Appl Physiol*, vol. 37, no. 2, pp. 247–8, 1974.

[309] J. P. Reed, B. K. Schilling, and Z. Murlasits, "Acute neuromuscular and metabolic responses to concurrent endurance and resistance exercise," *J Strength Cond Res*, vol. 27, no. 3, pp. 793–801, 2013.

[310] S. Brooks, M. E. Nevill, L. Meleagros, H. K. Lakomy, G. M. Hall, S. R. Bloom, and C. Williams, "The hormonal responses to repetitive brief maximal exercise in humans," *Eur J Appl Physiol Occup Physiol*, vol. 60, no. 2, pp. 144–8, 1990.

[311] N. P. Walsh *et al.*, "Position statement. part one: Immune function and exer-

cise," *Exerc Immunol Rev*, vol. 17, pp. 6–63, 2011.

[312] I. J. Elenkov and G. P. Chrousos, "Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity," *Ann N Y Acad Sci*, vol. 966, pp. 290–303, 2002.

[313] T. A. Butterfield, T. M. Best, and M. A. Merrick, "The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair," *J Athl Train*, vol. 41, no. 4, pp. 457–65, 2006.

[314] F. Zaldivar *et al.*, "Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes," *J Appl Physiol*, vol. 100, no. 4, pp. 1124–1133, 2006.

[315] B. K. Pedersen, A. Steensberg, and P. Schjerling, "Muscle-derived interleukin-6: possible biological effects," *J Physiol*, vol. 536, no. 2, pp. 329–337, 2001.

[316] B. K. Pedersen and M. A. Febbraio, "Muscles, exercise and obesity: skeletal muscle as a secretory organ," *Nat Rev Endocrinol*, vol. 8, no. 8, pp. 457–65, 2012.

[317] E. F. Coyle, "Substrate utilization during exercise in active people," *Am J Clin Nutr*, vol. 61, no. 4 Suppl, pp. 968S–979S, 1995.

[318] C. W. Nicholas, C. Williams, H. K. Lakomy, G. Phillips, and A. Nowitz, "Influence of ingesting a carbohydrate-electrolyte solution on endurance capacity during intermittent, high-intensity shuttle running," *J Sports Sci*, vol. 13, no. 4, pp. 283–90, 1995.

[319] G. C. Gaitanos, C. Williams, L. H. Boobis, and S. Brooks, "Human muscle metabolism during intermittent maximal exercise," *J Appl Physiol*, vol. 75, no. 2, pp. 712–9, 1993.

[320] R. A. Robergs *et al.*, "Muscle glycogenolysis during differing intensities of weight-resistance exercise," *J Appl Physiol*, vol. 70, no. 4, pp. 1700–6, 1991.

[321] K. Häkkinen and A. Pakarinen, "Acute hormonal responses to heavy resistance exercise in men and women at different ages," *Int J Sports Med*, vol. 16, no. 8, pp. 507–13, 1995.

[322] P. J. Abernethy, "Influence of acute endurance activity on isokinetic strength," *J Strength Cond Res*, vol. 7, no. 3, pp. 141–146, 1993.

[323] J. Helgerud, G. Rodas, O. J. Kemi, and J. Hoff, "Strength and endurance in elite football players," *Int J Sports Med*, vol. 32, no. 9, pp. 677–82, 2011.

[324] W. G. Hopkins, S. W. Marshall, A. M. Batterham, and J. Hanin, "Progressive statistics for studies in sports medicine and exercise science," *Med Sci Sports Exerc*, vol. 41, no. 1, pp. 3–13, 2009.

[325] M. R. McGuigan, S. J. Cormack, and N. D. Gill, "Strength and power profiling of athletes: Selecting tests and how to use the information for program design," *Strength & Conditioning Journal*, vol. 35, no. 6, pp. 7–14, 2013.

[326] M. S. Chelly, M. Fathloun, N. Cherif, M. Ben Amar, Z. Tabka, and E. Van Praagh, "Effects of a back squat training program on leg power, jump, and sprint performances in junior soccer players," *J Strength Cond Res*, vol. 23, no. 8, pp. 2241–9, 2009.

[327] B. R. Ronnestad, N. H. Kvamme, A. Sunde, and T. Raastad, "Short-term effects of strength and plyometric training on sprint and jump performance in professional soccer players," *J Strength Cond Res*, vol. 22, no. 3, pp. 773–80, 2008.

[328] D. Baker and S. Nance, "The relation between running speed and measures of strength and power in professional rugby league players," *J Strength Cond Res*, vol. 13, no. 3, pp. 230–235, 1999.

[329] R. Meir, R. Newton, E. Curtis, M. Fardell, and B. Butler, "Physical fitness qualities of professional rugby league football players: determination of positional differences," *J Strength Cond Res*, vol. 15, no. 4, pp. 450–8, 2001.

[330] D. Bishop, S. Lawrence, and M. Spencer, "Predictors of repeated-sprint ability in elite female hockey players," *J Sci Med Sport*, vol. 6, no. 2, pp. 199–209, 2003.

[331] D. Ferrari Bravo, F. M. Impellizzeri, E. Rampinini, C. Castagna, D. Bishop, and U. Wisløff, "Sprint vs. interval training in football," *Int J Sports Med*, vol. 29, no. 8, pp. 668–74, 2008.

[332] R. Starkie, S. R. Ostrowski, S. Jauffred, M. Febbraio, and B. K. Pedersen, "Exercise and il-6 infusion inhibit endotoxin-induced TNF-alpha production in humans," *FASEB J*, vol. 17, no. 8, pp. 884–6, 2003.

[333] J. A. Hawley, "Adaptations of skeletal muscle to prolonged, intense endurance training," *Clin Exp Pharmacol Physiol*, vol. 29, no. 3, pp. 218–222, 2002.

[334] T. R. Lundberg, R. Fernandez-Gonzalo, T. Gustafsson, and P. A. Tesch, "Aerobic exercise alters skeletal muscle molecular responses to resistance exercise," *Med Sci Sports Exerc*, vol. 44, no. 9, pp. 1680–8, 2012.

[335] H. L. Eley, S. T. Russell, and M. J. Tisdale, "Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor, and angiotensin ii by beta-hydroxy-beta-methylbutyrate," *Am J Physiol Endocrinol Metab*, vol. 295, no. 6, pp. E1409–16, 2008.

[336] D. L. Williamson, S. R. Kimball, and L. S. Jefferson, "Acute treatment with TNF-alpha attenuates insulin-stimulated protein synthesis in cultures of C2C12 myotubes through a MEK1-sensitive mechanism," *Am J Physiol Endocrinol Metab*, vol. 289, no. 1, pp. E95–104, 2005.

[337] J. S. Griewe, B. Cheng, D. C. Rubin, K. E. Yarasheski, and C. F. Semekovich, "Resistance exercise decreases skeletal muscle tumor necrosis factor ? in frail elderly humans," *FASEB J*, vol. 15, no. 2, pp. 475–482, 2001.

[338] B. Kendall and R. Eston, "Exercise-induced muscle damage and the potential protective role of estrogen," *Sports Med*, vol. 32, no. 2, pp. 103–23, 2002.

[339] P. M. Clarkson and M. J. Hubal, "Are women less susceptible to exercise-induced muscle damage?" *Curr Opin Clin Nutr Metab Care*, vol. 4, no. 6, pp. 527–531, 2001.

[340] N. Stupka, S. Lowther, K. Chorneyko, J. M. Bourgeois, C. Hogben, and M. A. Tarnopolsky, "Gender differences in muscle inflammation after eccentric exercise," *J Appl Physiol (1985)*, vol. 89, no. 6, pp. 2325–32, 2000.

[341] H. C. Dreyer, S. Fujita, J. G. Cadenas, D. L. Chinkes, E. Volpi, and B. B. Rasmussen, "Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle," *J Physiol*, vol. 576, no. 2, pp. 613–624, 2006.

[342] D. Camera, J. Edge, M. Short, J. Hawley, and V. Coffey, "Early time course of Akt phosphorylation after endurance and resistance exercise." *Med Sci Sports Exerc*, vol. 42, no. 10, pp. 1843–1852, 2010.

[343] M. J. Gibala, S. L. McGee, A. P. Garnham, K. F. Howlett, R. J. Snow, and M. Hargreaves, "Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1α in human skeletal muscle," *J Appl Physiol*, vol. 106, no. 3, pp. 929–934, 2009.

[344] J. R. Dent, J. A. Edge, E. Hawke, C. McMahon, and T. Mundel, "Sex differences in acute translational repressor 4E-BP1 activity and sprint performance in response to repeated-sprint exercise in team sport athletes," *J Sci Med Sport*, (In press).

[345] V. G. Coffey, Z. Zhong, A. Shield, B. J. Canny, A. V. Chibalin, J. R. Zierath, and J. A. Hawley, "Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans," *FASEB J*, 2005.

[346] P. C. Heinrich, I. Behrmann, S. Haan, H. M. Hermanns, G. Muller-Newen, and F. Schaper, "Principles of interleukin (IL)-6-type cytokine signalling and its regulation," *Biochem J*, vol. 374, no. Pt 1, pp. 1–20, 2003.

[347] K. Wang, C. Wang, F. Xiao, H. Wang, and Z. Wu, "JAK2/STAT2/STAT3 are required for myogenic differentiation," *J Biol Chem*, vol. 283, no. 49, pp. 34 029–36, 2008.

[348] M. Buchheit, A. Mendez-Villanueva, G. Delhomel, M. Brughelli, and S. Ahmaidi, "Improving repeated sprint ability in young elite soccer players: Repeated shuttle sprints vs. explosive strength training," *J Strength Cond Res*, vol. 24, no. 10, pp. 2715–2722, 2010.

[349] A. J. Murphy, R. G. Lockie, and A. J. Coutts, "Kinematic determinants of early acceleration in field sport athletes," *J Sports Sci Med*, vol. 2, no. 4, pp. 144–50, 2003.

[350] J. Wahren, P. Felig, G. Ahlborg, and L. Jorfeldt, "Glucose metabolism during leg exercise in man," *J Clin Invest*, vol. 50, no. 12, pp. 2715–25, 1971.

[351] B. C. Bergman, M. A. Horning, G. A. Casazza, E. E. Wolfel, G. E. Butterfield, and G. A. Brooks, "Endurance training increases gluconeogenesis during rest and exercise in men," *Am J Physiol Endocrinol Metab*, vol. 278, no. 2, pp. E244–51, 2000.

[352] A. R. Coggan, W. M. Kohrt, R. J. Spina, D. M. Bier, and J. O. Holloszy, "Endurance training decreases plasma glucose turnover and oxidation during moderate-intensity exercise in men," *J Appl Physiol*, vol. 68, no. 3, pp. 990–6, 1990.

[353] C. M. Donovan and K. D. Sumida, "Training enhanced hepatic gluconeogenesis: the importance for glucose homeostasis during exercise," *Med Sci Sports Exerc*, vol. 29, no. 5, pp. 628–34, 1997.

[354] C. A. Emhoff, L. A. Messonnier, M. A. Horning, J. A. Fattor, T. J. Carlson, and G. A. Brooks, "Gluconeogenesis and hepatic glycogenolysis during exercise at the lactate threshold," *J Appl Physiol*, vol. 114, no. 3, pp. 297–306, 2013.

[355] J. K. Trimmer, J. M. Schwarz, G. A. Casazza, M. A. Horning, N. Rodriguez, and G. A. Brooks, "Measurement of gluconeogenesis in exercising men by mass isotopomer distribution analysis," *J Appl Physiol*, vol. 93, no. 1, pp. 233–41, 2002.

[356] L. Al-Khalili, K. Bouzakri, S. Glund, F. Lonnqvist, H. A. Koistinen, and A. Krook, "Signaling specificity of interleukin-6 action on glucose and lipid metabolism in skeletal muscle," *Mol Endocrinol*, vol. 20, no. 12, pp. 3364–75, 2006.

[357] P. C. Geiger, C. Hancock, D. C. Wright, D. H. Han, and J. O. Holloszy, "Il-6 increases muscle insulin sensitivity only at superphysiological levels," *Am J Physiol Endocrinol Metab*, vol. 292, no. 6, pp. E1842–6, 2007.

[358] I. Nieto-Vazquez, S. Fernandez-Veledo, C. de Alvaro, and M. Lorenzo, "Dual role of interleukin-6 in regulating insulin sensitivity in murine skeletal muscle," *Diabetes*, vol. 57, no. 12, pp. 3211–21, 2008.

[359] M. Gleeson, "Interleukins and exercise," *J Physiol*, vol. 529, no. 1, p. 1, 2000.

[360] G. Smith *et al.*, "Clozapine directly increases insulin and glucagon secretion from islets: Implications for impairment of glucose tolerance," *Schizophr Res*, no. 0, 2014.

[361] M. S. Harbuz, A. Stephanou, N. Sarlis, and S. L. Lightman, "The effects of recombinant human interleukin (IL)-1 alpha, IL-1 beta or IL-6 on hypothalamo-pituitary-adrenal axis activation," *J Endocrinol*, vol. 133, no. 3, pp. 349–55, 1992.

[362] M. Wojewoda *et al.*, "Running performance at high running velocities is impaired but v'o((2)max) and peripheral endothelial function are preserved in il-6(-)/(-) mice," *PLoS One*, vol. 9, no. 2, p. e88333, 2014.

[363] K. Häkkinen, A. Pakarinen, and M. Kallinen, "Neuromuscular adaptations and serum hormones in women during short-term intensive strength training," *Eur J Appl Physiol*, vol. 64, no. 2, pp. 106–111, 1992.

[364] D. G. Sale, "Neural adaptation to resistance training," *Med Sci Sports Exerc*, vol. 20, no. 5 Suppl, pp. S135–45, 1988.

[365] P. Aagaard, E. B. Simonsen, J. L. Andersen, P. Magnusson, and P. Dyhre-Poulsen, "Increased rate of force development and neural drive of human skeletal muscle following resistance training," *J Appl Physiol*, vol. 93, no. 4, pp. 1318–26, 2002.

[366] A. R. Creer, M. D. Ricard, R. K. Conlee, G. L. Hoyt, and A. C. Parcell, "Neural, metabolic, and performance adaptations to four weeks of high intensity sprint-interval training in trained cyclists," *Int J Sports Med*, vol. 25, no. 2, pp. 92–8, 2004.

[367] T. Moritani and M. Muro, "Motor unit activity and surface electromyogram power spectrum during increasing force of contraction," *Eur J Appl Physiol*, vol. 56, no. 3, pp. 260–265, 1987.

[368] D. Pette and R. S. Staron, "Transitions of muscle fiber phenotypic profiles," *Histochem Cell Biol*, vol. 115, no. 5, pp. 359–72, 2001.

[369] G. Campos *et al.*, "Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones," *Eur J Appl Physiol*, vol. 88, no. 1-2, pp. 50–60, 2002.

[370] N. Wang, R. S. Hikida, R. S. Staron, and J.-A. Simoneau, "Muscle fiber types of women after resistance training–quantitative ultrastructure and enzyme activity," *Pfluegers Arch*, vol. 424, no. 5-6, pp. 494–502, 1993.

[371] G. R. Adams, B. M. Hather, K. M. Baldwin, and G. A. Dudley, "Skeletal muscle myosin heavy chain composition and resistance training," *J Appl Physiol*, vol. 74, no. 2, pp. 911–915, 1993.

[372] B. Dawson, M. Fitzsimons, S. Green, C. Goodman, M. Carey, and K. Cole, "Changes in performance, muscle metabolites, enzymes and fibre types after short sprint training," *Eur J Appl Physiol*, vol. 78, no. 2, pp. 163–169, 1998.

[373] C. Putman, X. Xu, E. Gillies, I. MacLean, and G. Bell, "Effects of strength, endurance and combined training on myosin heavy chain content and fibre-type distribution in humans," *Eur J Appl Physiol*, vol. 92, no. 4-5, pp. 376–384, 2004.

[374] R. G. Lockie, A. J. Murphy, T. J. Knight, and X. A. Janse de Jonge, "Factors that differentiate acceleration ability in field sport athletes," *J Strength Cond Res*, vol. 25, no. 10, pp. 2704–14, 2011.

[375] A. Castillo-Rodriguez, J. C. Fernandez-Garcia, J. L. Chinchilla-Minguet, and E. A. Carnero, "Relationship between muscular strength and sprints with changes of direction," *J Strength Cond Res*, vol. 26, no. 3, pp. 725–32, 2012.

[376] M. Brughelli, J. Cronin, G. Levin, and A. Chaouachi, "Understanding change of direction ability in sport: a review of resistance training studies," *Sports Med*, vol. 38, no. 12, pp. 1045–63, 2008.

[377] T. Reilly, A. M. Williams, A. Nevill, and A. Franks, "A multidisciplinary approach to talent identification in soccer," *J Sports Sci*, vol. 18, no. 9, pp. 695–702, 2000.

[378] T. Hazell, R. MacPherson, B. Gravelle, and P. Lemon, "10 or 30-s sprint interval training bouts enhance both aerobic and anaerobic performance," *Eur J Appl Physiol*, vol. 110, no. 1, pp. 153–160, 2010.

[379] R. Q. Landers-Ramos, N. T. Jenkins, E. E. Spangenburg, J. M. Hagberg, and S. J. Prior, "Circulating angiogenic and inflammatory cytokine responses to acute aerobic exercise in trained and sedentary young men," *Eur J Appl Physiol*, 2014.

[380] D. B. Pyne, "Exercise-induced muscle damage and inflammation: a review," *Aust J Sci Med Sport*, vol. 26, no. 3-4, pp. 49–58, 1994.

[381] M. P. McHugh, "Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise," *Scand J Med Sci Sports*, vol. 13, no. 2, pp. 88–97, 2003.

[382] S. Glund *et al.*, "Interleukin-6 directly increases glucose metabolism in resting

human skeletal muscle," *Diabetes*, vol. 56, no. 6, pp. 1630–7, 2007.

[383] Z. Yu *et al.*, "Differences between human plasma and serum metabolite profiles," *PLoS One*, vol. 6, no. 7, p. e21230, 2011.

[384] P. T. Reidy *et al.*, "Protein blend ingestion following resistance exercise promotes human muscle protein synthesis," *J Nutr*, vol. 143, no. 4, pp. 410–416, 2013.

[385] V. G. Coffey *et al.*, "Nutrient provision increases signalling and protein synthesis in human skeletal muscle after repeated sprints," *Eur J Appl Physiol*, vol. 111, no. 7, pp. 1473–83, 2011.

[386] D. M. Camera, D. W. West, S. M. Phillips, T. Rerecich, T. Stellingwerff, J. A. Hawley, and V. G. Coffey, "Protein ingestion increases myofibrillar protein synthesis after concurrent exercise," *Med Sci Sports Exerc*, 2014.

[387] DBL. Octreotide injection datasheet 8 march 2012. 3 http://www.medsafe.govt.nz/profs/datasheet/d/dblOctreotideinj.pdf (accessed: 01 June 2014)

Appendix

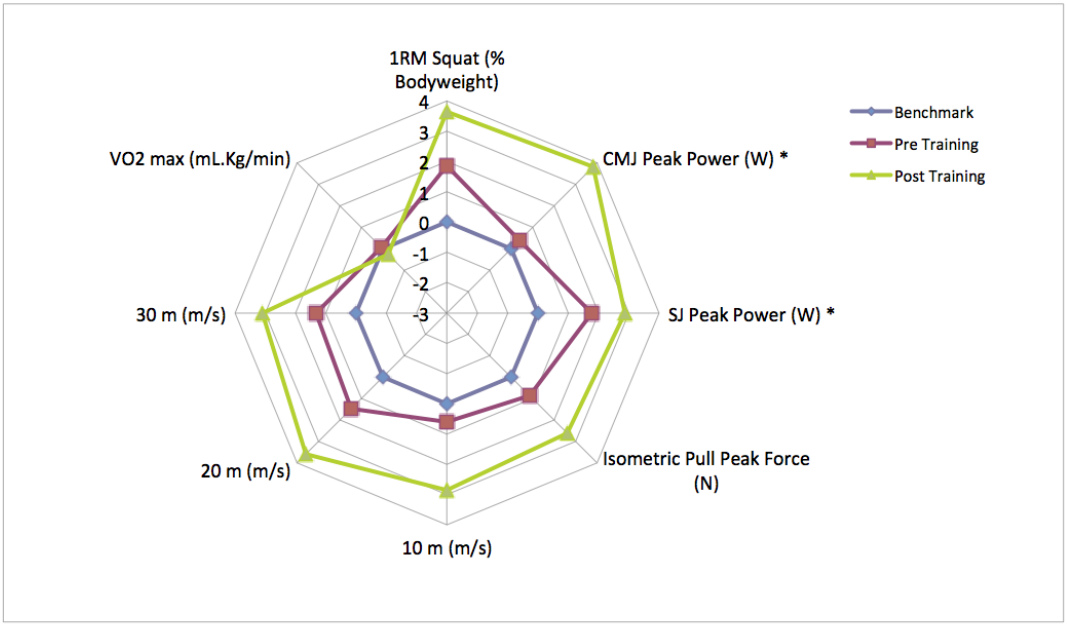
Example participant performance feedback: radar plot

Performance Results Pre and Post 4 wk of Concurrent Repeated-Sprint and Resistance Training

Participant: 12

Test	Absolute			Div Bodyweight		
	Pre	Post	% Change	Pre	Post	% Change
1RM Squat (kg)	75	90	20%	1.26	1.51	17%
CMJ Peak Power (W)	2448	3068	25%	41.16	51.46	20%
SJ Peak Power (W)	2750	2985	9%	46.23	50.07	8%
Iso Pull Peak Force (N)	1584.7	1857.5	17%			
VO2 max (mL.Kg/min)	45.2	44	-3%			

Sprint	Time (Sec)				Velocity (m/s)		
	Dist	Pre	Post	% Change	Pre	Post	% Change
	10 m	1.92	1.77	-8%	5.22	5.67	8%
	20 m	3.26	3.08	-6%	6.13	6.49	6%
	30 m	4.6	4.33	-6%	6.53	6.92	6%



Test	Pre	Post	Benchmark	SD	Benchmark	Z Score Pre	Z Score Post
1RM Squat (% Bodyweight)	126%	151%	1	0.14	0	1.86	3.64
CMJ Peak Power (W) *	41.2	51.5	40	3	0	0.39	3.82
SJ Peak Power (W) *	46.2	50.1	40	3.5	0	1.78	2.88
Isometric Pull Peak Force (N)	1585	1858	1450	156	0	0.86	2.61
10 m (m/s)	5.22	5.67	5.1	0.2	0	0.60	2.85
20 m (m/s)	6.13	6.49	5.88	0.17	0	1.47	3.59
30 m (m/s)	6.53	6.92	6.24	0.22	0	1.32	3.09
VO2 max (mL.Kg/min)	45.2	44.0	45	3.9	0	0.05	-0.26

Z score = (athletes score - benchmark)/benchmark standard deviation

Note: * Relative to Bodyweight

Figure A.1: Example participant performance feedback: radar plot and pre-post performance change following 4 weeks of repeated-sprint and resistance training.