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**PHYSIOLOGICAL AND  
MOLECULAR RESPONSES TO  
CONCURRENT TRAINING IN  
ENDURANCE-TRAINED  
ATHLETES**

L J EDDENS

PhD

2019

**PHYSIOLOGICAL AND  
MOLECULAR RESPONSES TO  
CONCURRENT TRAINING IN  
ENDURANCE-TRAINED  
ATHLETES**

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A thesis submitted in partial fulfilment of  
the requirements of the University of  
Northumbria at Newcastle for the degree  
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Health and Life Sciences and in  
collaboration with GlaxoSmithKline

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## ***Abstract***

Concurrent training describes the combination of both endurance and strength stimuli within a coherent paradigm. An attenuation in strength, power, and hypertrophy can result from this model of training, in comparison to that resulting from strength only; a phenomenon termed an ‘interference effect’. Rather than adopt a myopic view of the concurrent training paradigm, whereby the addition of an endurance stimulus is fatal to strength-based training outcomes, it is of interest to better understand the response to a concurrent exercise stimulus and elucidate the response to manipulating training variables. Given this, the objectives of this thesis were to investigate and draw conclusions on the short-term response to a strenuous concurrent stimulus (Chapter 4), in addition to the effect of manipulating the programme variables of exercise sequence (Chapter 5) and endurance exercise intensity (Chapters 6 and 7) on strength and endurance-related outcomes.

The findings of this thesis indicate concurrent training stimuli to provide a relatively modest level of muscle damage, potentially owing to the order of the two exercise modes, with this programme variable of intra-session exercise sequence affecting improvements in lower-body dynamic strength during a concurrent training programme. Further, the data do not support the premise of a molecular interference effect amongst an endurance-trained phenotype, nor a role for the variable of endurance exercise intensity to modify the molecular response to concurrent stimuli, regardless of training status, or performance outcomes following a short-term concurrent training programme.

With regards to practical applications, while an endurance-resistance exercise order might limit muscle damage, the alternate sequence is beneficial for lower-body strength development. Individuals limited by time, such that they must train concurrently with minimal relief between modes of exercise, should adopt a resistance-endurance exercise order to promote strength adaptation across a training programme. Finally, providing it is work- and duration-matched, endurance exercise intensity can be manipulated without detriment to strength performance, amongst endurance trained athletes.

## ***Contents***

<b>Abstract .....</b>	<b>I</b>
<b>Contents .....</b>	<b>II</b>
<b>List of Tables .....</b>	<b>IX</b>
<b>List of Figures .....</b>	<b>X</b>
<b>List of Abbreviations .....</b>	<b>XIII</b>
<b>Publications and Conference Communications .....</b>	<b>XVII</b>
<b>Acknowledgements .....</b>	<b>XVIII</b>
<b>Author's Declaration .....</b>	<b>XIX</b>
<b>Chapter 1</b>	
<b>Introduction.....</b>	<b>1</b>
1.1 Introduction .....	2
1.2 Aims and objectives .....	5
<b>Chapter 2</b>	
<b>Literature review .....</b>	<b>7</b>
2.1 Literature review .....	8
2.2 Exercise stress specificity .....	9
2.2.1 <i>Responses to resistance training stress</i> .....	12
2.2.2 <i>Responses to endurance training stress</i> .....	15
2.2.3 <i>Responses to concurrent training stress</i> .....	17
2.2.4 <i>Role of protein to aid recovery from training stress</i> .....	20
2.2.5 <i>Adaptations to strength training stress</i> .....	21
2.2.6 <i>Adaptations to endurance training stress</i> .....	23
2.3 The Interference Phenomenon .....	26
2.3.1 <i>The chronic and acute hypotheses</i> .....	27
2.3.2 <i>Role of programme variables</i> .....	28
2.3.2.1 <i>Sequencing of exercise</i> .....	28

2.3.2.2 <i>Intensity of exercise</i> .....	31
2.3.2.3 <i>Frequency of exercise</i> .....	33
2.4 Proposed mechanisms for the interference phenomenon .....	35
2.4.1 <i>Fatigue mechanisms</i> .....	35
2.4.2 <i>Neuromuscular factors</i> .....	38
2.4.3 <i>Molecular factors</i> .....	39
2.4.4 <i>Training status</i> .....	42
2.5 Summary .....	43
<b>Chapter 3</b>	
<b>General methods</b> .....	<b>45</b>
3.1 General methods .....	46
3.2 Ethical approval .....	46
3.3 Participants .....	46
3.4 Performance measures .....	47
3.4.1 <i>Assessment of peak oxygen uptake</i> .....	47
3.4.2 <i>Counter-movement jump</i> .....	48
3.4.3 <i>Maximal strength testing</i> .....	48
3.5 Acute exercise stimulus .....	49
3.5.1 <i>Exercise and dietary control</i> .....	49
3.5.2 <i>Resistance exercise stimulus</i> .....	50
3.5.3 <i>Endurance exercise stimulus</i> .....	50
3.5.4 <i>Muscle sampling</i> .....	51
3.6 Biochemical analyses .....	54
3.6.1 <i>Blood lactate analysis</i> .....	54
3.6.2 <i>Muscle analysis</i> .....	54
3.7 Statistical analyses .....	56

## Chapter 4

<b>The acute response to a muscle-damaging concurrent training stimulus and efficacy of protein supplementation to aid recovery .....</b>	<b>57</b>
4.1 Abstract .....	58
4.2 Introduction .....	59
4.3 Methods .....	61
4.3.1 <i>Design</i> .....	61
4.3.2 <i>Participants</i> .....	63
4.3.3 <i>Procedures</i> .....	63
4.3.3.1 <i>Nutritional supplement</i> .....	63
4.3.3.2 <i>Dietary control</i> .....	65
4.3.3.3 <i>Assessment of peak oxygen uptake</i> .....	65
4.3.3.4 <i>Muscle damaging concurrent exercise</i> .....	65
4.3.3.5 <i>Maximal voluntary contraction</i> .....	67
4.3.3.6 <i>Counter-movement jump</i> .....	68
4.3.3.7 <i>16.1 km time trial</i> .....	68
4.3.3.8 <i>Muscle soreness assessment</i> .....	69
4.3.3.9 <i>Blood sampling and analysis</i> .....	70
4.3.3.10 <i>Statistical analysis</i> .....	71
4.4 Results .....	71
4.4.1 <i>Performance measures</i> .....	72
4.4.1.1 <i>Performance measures</i> .....	72
4.4.1.2 <i>Muscle soreness</i> .....	74
4.4.1.3 <i>Serum proteins</i> .....	75
4.4.1.4 <i>Body mass and supplementation</i> .....	76
4.5 Discussion .....	76
4.6 Summary .....	82

## Chapter 5

### **The role of acute exercise sequence in the provision of the interference effect: a**

<b>systematic review with meta-analysis .....</b>	<b>84</b>
5.1 Abstract .....	85
5.2 Introduction .....	86
5.3 Methods .....	88
5.3.1 <i>Criteria for study eligibility: studies and subjects</i> .....	88
5.3.2 <i>Information sources and search strategy</i> .....	90
5.3.3 <i>Study selection and data processing</i> .....	91
5.3.4 <i>Quality assessment</i> .....	92
5.4 Results .....	93
5.4.1 <i>Study characteristics</i> .....	96
5.4.2 <i>Publication bias and inconsistency</i> .....	96
5.4.3 <i>Intervention effects and pooled analyses</i> .....	98
5.5 Discussion .....	106
5.6 Summary .....	110

## Chapter 6

### **The acute interference effect and the role of exercise intensity .....**

6.1 Abstract .....	113
6.2 Introduction .....	114
6.3 Methods .....	116
6.3.1 <i>Design</i> .....	116
6.3.2 <i>Participants</i> .....	117
6.3.3 <i>Procedures</i> .....	117
6.3.3.1 <i>Preliminary testing</i> .....	117
6.3.3.2 <i>Assessment of peak oxygen uptake</i> .....	118
6.3.3.3 <i>Maximal strength testing</i> .....	118
6.3.4 <i>Acute exercise stimulus</i> .....	118

6.3.4.1 Exercise and dietary control .....	118
6.3.4.2 Resistance exercise stimulus .....	118
6.3.4.3 Endurance exercise stimulus .....	118
6.3.4.4 Muscle sampling .....	119
6.3.4.5 Blood lactate sampling .....	119
6.3.5 Analyses .....	119
6.3.5.1 Muscle analysis .....	119
6.3.5.2 Statistical analysis .....	119
6.4 Results .....	120
6.4.1 Physiological response .....	120
6.4.2 Signalling response .....	121
6.4.2.1 AMPK pathway .....	121
6.4.2.2 mTOR pathway .....	123
6.5 Discussion .....	125
6.6 Summary .....	131

## **Chapter 7**

<b>The role of endurance exercise intensity during short-term concurrent training .....</b>	<b>132</b>
7.1 Abstract .....	133
7.2 Introduction .....	134
7.3 Methods .....	137
7.3.1 Design .....	137
7.3.2 Participants .....	138
7.3.3 Preliminary procedures .....	139
7.3.3.1 Preliminary testing .....	139
7.3.3.2 Graded exercise test .....	139
7.3.3.3 Assessment of peak oxygen uptake .....	139

7.3.3.4	<i>Body composition</i>	139
7.3.3.5	<i>Counter-movement jump</i>	140
7.3.3.6	<i>5 min time trial</i>	140
7.3.3.7	<i>Maximal strength testing</i>	141
7.3.4	<i>Single concurrent exercise stimulus</i>	141
7.3.4.1	<i>Exercise and dietary control</i>	141
7.3.4.2	<i>Resistance exercise stimulus</i>	141
7.3.4.3	<i>Endurance exercise stimulus</i>	141
7.3.4.4	<i>Muscle sampling</i>	141
7.3.4.5	<i>Blood sampling</i>	141
7.3.5	<i>Training intervention</i>	142
7.3.5.1	<i>Resistance training</i>	143
7.3.5.2	<i>Endurance training</i>	144
7.3.5.3	<i>Training load quantification</i>	145
7.3.5.4	<i>Exercise and dietary control</i>	146
7.3.6	<i>Analyses</i>	146
7.3.6.1	<i>Muscle analysis</i>	146
7.3.6.2	<i>Statistical analysis</i>	146
7.4	<i>Results</i>	147
7.4.1	<i>Training compliance</i>	147
7.4.2	<i>Training load</i>	147
7.4.2.1	<i>Session load and training impulse score</i>	147
7.4.3	<i>Body composition</i>	148
7.4.3.1	<i>Body composition</i>	148
7.4.4	<i>Performance measures</i>	149
7.4.4.1	<i>Maximal strength</i>	149
7.4.4.2	<i>Cycling specific performance</i>	153

7.4.4.3 Counter-movement jump performance .....	154
7.4.4.4 AMPK pathway .....	154
7.4.4.5 mTOR pathway .....	155
7.5 Discussion .....	157
7.6 Summary .....	163
<b>Chapter 8</b>	
<b>General discussion .....</b>	<b>165</b>
8.1 Aims and objectives of this thesis .....	166
8.2 Chapter reviews .....	166
8.2.1 Chapter 4 .....	166
8.2.2 Chapter 5 .....	167
8.2.3 Chapter 6 .....	168
8.2.4 Chapter 7 .....	169
8.3 General discussion .....	170
8.3.1 Role of programme variables .....	170
8.3.1.1 Sequencing of exercise .....	170
8.3.1.2 Intensity of exercise .....	172
8.3.2 Mechanisms relating to the interference phenomenon .....	174
8.3.2.1 Molecular factors .....	174
8.3.2.2 Training status .....	176
8.4 Practical applications .....	177
8.5 Limitations .....	179
8.6 Future research .....	180
8.7 Conclusions .....	182
<b>References .....</b>	<b>185</b>

## *List of Tables*

- Table 4.1.** Nutritional content of the supplements.
- Table 4.2.** High intensity simulated cycling road race; sprint frequency/duration and work to rest ratio composition.
- Table 4.3.** Baseline demographic descriptive data.
- Table 4.4.** Daily prescribed energy intake.
- Table 4.5.** Participant selection of perceived treatment condition at the conclusion of study participation.
- Table 5.1.** PubMed search strategy performed on 5<sup>th</sup> February 2016. The entry column details specific search terms entered into databases, to identify research aligned to the PICO search strategy.
- Table 5.2.** Quality assessment ‘QualSyst’.
- Table 5.3.** Characteristics of the individual studies included in the meta-analysis.
- Table 5.4.** Individual study results included in the meta-analysis.
- Table 6.1.** Physiological response to the MIC and work-matched HIIC protocols.
- Table 6.2.** Training load quantification of the MIC and work-matched HIIC protocols.
- Table 7.1** Detail of the 8 wk resistance training programme.
- Table 7.2.** Baseline and pre to post-training change in body composition parameters for the MIC and HIIC groups.
- Table 7.3.** Pre to post-training change in aerobic thresholds and  $\dot{V}O_{2peak}$  for the MIC and HIIC groups.

## *List of Figures*

- Figure 2.1.** An illustration of the timecourse of changes from baseline in mRNA expression, protein content, and exercise performance following acute and chronic exercise training.
- Figure 2.2.** The strength-endurance continuum of different sporting events and disciplines, relative to exercise mode characteristics, event duration and energy metabolism.
- Figure 2.3.** An illustration of the key pathways that regulate muscle fibre size.
- Figure 2.4.** An illustration of the timecourse leading from acute endurance exercise, through to mitochondrial biogenesis.
- Figure 3.1.** Schematic of the work and duration-matched MIC and HIIC protocols, forming the endurance exercise element of the acute exercise stimulus.
- Figure 3.2.** Schematic of the single exercise stimuli.
- Figure 3.3.** The rest and 3 h post-resistance exercise biopsy locations.
- Figure 3.4.** A depiction of the nitrocellulose membrane placement for the imaging process.
- Figure 3.5.** An illustrative JPEG generated by the GeneSys imaging software.
- Figure 4.1.** Study schematic.
- Figure 4.2.** A blinded single serving of supplemental beverage.
- Figure 4.3.** The experimental set up for the CMJ and MVC protocols.
- Figure 4.4.** The experimental set up for the 16.1 km time trial protocol.
- Figure 4.5.** Isometric maximal voluntary contraction response to the concurrent exercise protocol.
- Figure 4.6.** CMJ response to the concurrent exercise protocol.
- Figure 4.7.** Muscle soreness response to the concurrent exercise protocol.

- Figure 5.1.** Flow diagram of study screening process.
- Figure 5.2.** Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on lower-body dynamic strength.
- Figure 5.3.** Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on lower-body muscle hypertrophy.
- Figure 5.4.** Forest plot of the results of a fixed-effects meta-analysis shown as pooled mean differences with 95% CIs on lower-body static strength.
- Figure 5.5.** Forest plot of the results of a fixed-effects meta-analysis shown as pooled mean differences with 95% CIs on maximal aerobic capacity.
- Figure 5.6.** Forest plot of the results of a fixed-effects meta-analysis shown as pooled mean differences with 95% CIs on body fat %.
- Figure 6.1.** Mean response in phosphorylation of the AMPK signalling pathway in the RES, RES + MIC, and RES + HIIC conditions.
- Figure 6.2.** Mean response in phosphorylation of the mTOR signalling pathway in the RES, RES + MIC, and RES + HIIC conditions.
- Figure 7.1.** Study schematic.
- Figure 7.2.** The experimental set up for a supervised training session.
- Figure 7.3.** Prescribed (laboratory) and non-prescribed (external) training load for endurance activity performed across the 8 wk training intervention.
- Figure 7.4.** 5-RM back-squat performance across the intervention period.
- Figure 7.5.** 5-RM split-squat performance across the intervention period.
- Figure 7.6.** 5-RM calf-raise performance across the intervention period.

**Figure 7.7.** Mean 5 min TT performance at pre and post- intervention.

**Figure 7.8.** Mean CMJ height at pre and post- intervention.

**Figure 7.9.** Mean response in phosphorylation of the AMPK signalling pathway.

**Figure 7.10.** Mean response in phosphorylation of the mTOR signalling pathway.

## *List of Abbreviations*

4E-BP1	4E binding protein 1
ACSM	American College of Sports Medicine
ADP	adenosine diphosphate
Akt	protein kinase B
AMP	adenosine monophosphate
AMPK	adenosine monophosphate activated protein kinase
ANOVA	analysis of variance
ATP	adenosine triphosphate
BF%	body fat percentage
CHO	carbohydrate
CK	creatine kinase
CMJ	counter-movement jump
CRP	C-reactive protein
CSA	cross-sectional area
d	day
DNA	deoxyribonucleic acid
DXA	dual-energy x-ray absorptiometry
eEF2	eukaryotic elongation factor 2
eIF4B	eukaryotic initiation factor 4B
eIF4E	eukaryotic initiation factor 4E
EIMD	exercise-induced muscle damage
ELISA	enzyme-linked immunosorbent assay
EMG	electromyography

END	endurance
ERK 1/2	extracellular signal-regulated kinase 1/2
g	gram
GSK-3 $\alpha/\beta$	glycogen synthase kinase-3 $\alpha/\beta$
h	hour
HIIC	high intensity interval cycling
HIIT	high intensity interval training
HSP27	heat shock protein 27
Hz	hertz
ISAK	International Society for the Advancement of Kinanthropometry
kcal	kilocalorie
kg	kilogram
km	kilometre
L	litre
Lac <sup>-</sup>	blood lactate concentrations
LB	lower-body
LT	lactate threshold
mg	milligram
MIC	moderate intensity cycling
MICT	moderate intensity continuous training
min	minute
mmol	millimole
MPS	myofibrillar protein synthesis
MRI	magnetic resonance imaging

mRNA	messenger ribonucleic acid
ms	millisecond
mTOR	mammalian target of rapamycin
MVC	maximal voluntary contraction
NFOR	non-functional overreaching
nm	nanometre
N·m	Newton metre
O <sub>2</sub>	oxygen
OTS	overtraining syndrome
p38	mitogen-activated protein kinase
p53	tumour protein p53
p70S6K	70-kDa S6 protein kinase
PGC-1 $\alpha$	peroxisome proliferator-activated receptor gamma, co activator 1 alpha
P <sub>i</sub>	inorganic phosphate
PRAS40	proline-rich Akt substrate of 40 kDa
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRO	protein
RER	respiratory exchange ratio
RES	resistance
RFD	rate of force development
RM	repetition maximum
RPE	rate of perceived exertion
rpS6	ribosomal protein S6
s	second

SD	standard deviation
STAT2	signal transducer and activator of transcription 2
TSC2	tuberous sclerosis complex 2
TT	time trial
UB	upper-body
$\dot{V}O_{2\text{peak}}$	peak oxygen uptake
$\dot{V}O_{2\text{max}}$	maximal oxygen uptake
W	watt
wk	week
yr	year

## ***Publications and Conference Communications***

Data within this thesis has been presented in the following peer reviewed publications and conference presentations:

Eddens, L., Browne, S., Stevenson, E. J., Sanderson, B., van Someren, K. and Howatson, G. (2017). The efficacy of protein supplementation during recovery from muscle-damaging concurrent exercise. *Applied Physiology, Nutrition, and Metabolism*, 42 (7), pp 716-24.

Eddens, L., van Someren, K. and Howatson, G. (2018). The role of intra-session exercise sequence in the interference effect: a systematic review with meta-analysis. *Sports Medicine*, 48 (1), pp 177-88.

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## ***Author's Declaration***

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others. The work was completed in collaboration with the GlaxoSmithKline Human Performance Laboratory.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted by the Faculty Ethics Committee on 11<sup>th</sup> May 2015, 2<sup>nd</sup> September 2015, and 10<sup>th</sup> March 2017, for the respective studies.

**I declare that the Word Count of this Thesis is 44,861 words.**

Name:

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# **1. INTRODUCTION**

## 1.1 Introduction

A training paradigm with a focus on increasing or maintaining the maximum force generated by the muscle can be defined as strength training (Knuttgén and Kraemer, 1987). Specifically, strength training is undertaken to elicit an improvement in the structure and the maximal tension of the muscle, assisting the neuromuscular system to generate force and overcome resistance (Hawley et al., 2014). Within this training paradigm, factors that include the type of resistance employed, exercise choice, rest interval, and the number of repetitions/sets, are vital to the goal of improving strength (Kraemer et al., 2002). In an acute context, this high-load, low-frequency bout of contractile activity activates and/or represses signalling pathways, resulting in an increase in myofibrillar protein content (Egan and Zierath, 2013, Phillips, 2014). Chronically, adaptations to successful strength training include improved neural recruitment patterns, increased muscle cross-sectional area (CSA), and modifications to muscle architecture, eliciting greater force generation of the muscle (Häkkinen, 1989, Kawakami et al., 1993, McCall et al., 1996, Milner-Brown et al., 1975).

Conversely, endurance training is characterised by high-frequency and low-load muscular contractions. Similar to strength training, the stimulus must be repeated within sessions of appropriate intensity, frequency, and duration, in order to elicit improvements in key parameters of aerobic function. Parameters determining aerobic capacity are motor unit recruitment by the central nervous system, the delivery of O<sub>2</sub> by the pulmonary and cardiovascular systems, and the ability of the working musculature to consume O<sub>2</sub> within the oxidative pathways (Hawley et al., 2014). Numerous cellular events occur in response to an endurance stimulus. An example of which are the cellular processes which result in the expression of mitochondrial proteins, and increased mitochondrial density over time (Lin et al., 2005). An increase in mitochondrial density is a key adaptation in skeletal muscle following endurance training, ultimately acting to regulate substrate metabolism by promoting greater fat oxidation and reducing carbohydrate oxidation (Holloszy and Coyle, 1984a).

Eliciting both strength and endurance adaptations in a simultaneous fashion would prove beneficial for athletes of many sporting disciplines. Training conducted with these objectives is termed concurrent training. This requirement to elicit adaptation in divergent physiology is often thought to be troublesome. The seminal work of Dr Robert Hickson presented an attenuation in strength improvement in the concurrent *vs.* the strength training group (Hickson, 1980); a finding described as the ‘interference of strength development’ and later phrased the ‘interference effect’ or ‘interference phenomenon’. Subsequent efforts to better understand and explain this incompatibility of combined strength and endurance training has resulted in mixed evidence, with data that has supported (Cadore et al., 2010, Dudley and Djamil, 1985, Häkkinen et al., 2003, Hennessy and Watson, 1994) and refuted (Chtara et al., 2008, Lundberg et al., 2013, McCarthy et al., 2002, Silva et al., 2012) the theory of an interference effect.

Given the necessity for numerous sporting populations to develop strength and aerobic capacity simultaneously, a significant demand has been placed upon the practice of effective concurrent training methods. This has naturally led to interest in the possible mechanisms underpinning the examples of interference and these purported mechanisms can be grouped into “acute” and “chronic” hypotheses. The chronic hypothesis simply contends that these divergent processes are antagonistic to one another, leading to sub-optimal adaptation at the skeletal muscle level, in comparison to those which would be achieved by performing either mode of training in isolation (Leveritt et al., 1999). Attempts to investigate this hypothesis have resulted in the observation of muscle fibre type transitions (Häkkinen et al., 2003, Kraemer et al., 1995, Sale et al., 1990b), muscle fibre hypertrophy (Kraemer et al., 1995, Nelson et al., 1990), motor unit recruitment (Häkkinen et al., 2003, Mikkola et al., 2012), and molecular responses to exercise (Coffey and Hawley, 2016, Lundberg et al., 2013). The acute hypothesis proposes that interference is not the result of maladaptation from combining divergent stimuli, but rather, that the quality of the strength stimulus is compromised by the concurrent endurance stimulus

(Leveritt et al., 1999). Efforts to explore this hypothesis have manipulated inter-session recovery i.e. duration between strength and endurance modes of exercise (Robineau et al., 2016, Sporer and Wenger, 2003b) and intra-session sequence i.e. the order of the concurrent stimulus (Cadore et al., 2013, Pinto et al., 2015).

Despite almost four decades since the seminal work in the field, much debate remains as to the existence or extent of the interference effect. A meta-analysis by Wilson et al. (2012) provided a quantitative approach to investigating the existence of the interference phenomenon, using data from 21 studies. Decrements in adaptation for strength, power and hypertrophy across a training programme were observed in the concurrent groups *vs.* the resistance training groups, however these responses were only significantly blunted for power (Wilson et al., 2012). Conversely, a recent review concluded experimental evidence for interference on muscle hypertrophy to be non-existent (Murach and Bagley, 2016), while some authors have reported concurrent training to augment muscle growth, but not strength, relative to resistance training alone (Lundberg et al., 2013, Lundberg et al., 2014, Mikkola et al., 2012). It is tempting to adopt a somewhat myopic view of the concurrent training paradigm, whereby the addition of an endurance stimulus is fatal to strength, hypertrophy, and/or power. Instead, it is of interest to better understand the effect of manipulating training variables, in search of an optimum adaptation for a given training load and performance demand. A particular challenge is presented by the multitude of training variables involved in a concurrent training paradigm. It is acknowledged that clarifying the potential role for these programme variables to manipulate the interference effect is a vast undertaking (Fyfe et al., 2014).

Beyond sports with a distinct rationale for concurrent training, endurance athletes are also considered to benefit from this training paradigm, primarily owing to improvements in economy of movement (Rønnestad and Mujika, 2014). Indeed, there are numerous data to support improved exercise economy amongst endurance athletes following concurrent training (Guglielmo et al., 2009, Hoff et al., 2002, Johnson et al.,

1997, Millet et al., 2002). Adding strength training to endurance activity has also been associated with improved power/speed at various lactate thresholds (Mikkola et al., 2007, Ronnestad et al., 2010, Taipale et al., 2013) and TT performance (Aagaard et al., 2011, Hickson et al., 1988, Koninckx et al., 2010), although this evidence is more equivocal than that of exercise economy (Ronnestad and Mujika, 2014). The majority of investigations of the topic of interference and concurrent training have employed untrained or recreationally active populations, with few authors observing trained or well-trained cohorts (Enright et al., 2015, Ronnestad et al., 2012). To this author's knowledge, there are no interventional data comparing concurrent *vs.* resistance training outcomes amongst trained endurance athletes. This is likely owing to the ethical considerations in allocating trained and competing endurance athletes to a resistance-only training group. However, this question also remains unexplored in an acute context, despite evidence of training status acting to modify the early molecular signalling responses to opposing exercise stimuli (Coffey et al., 2006).

## **1.2 Aims and objectives**

The primary focus of this thesis was to investigate the unique challenge presented by undertaking a concurrent training paradigm. This objective was explored both by way of observing the recovery profile following a strenuous concurrent exercise stimulus within the context of the acute hypothesis, and by way of assessing how pertinent training variables might act to present an interference effect in the context of the chronic hypothesis, or provide superior adaptive responses compared to an alternate model of concurrent training. As such, a programme of research was undertaken, encompassing four specific, but sequential aims;

1. To examine the recovery profile in response to a strenuous concurrent training stimulus, amongst an endurance trained cohort.

2. To assess whether intra-session concurrent exercise sequence modifies strength-based outcomes associated with the interference effect, following short-term concurrent training.
3. To investigate whether an interference effect is evident amongst an endurance-trained cycling cohort and whether endurance exercise intensity plays a role in moderating the potential effect.
4. To examine whether endurance exercise intensity modifies the adaptation in lower-body strength following short-term concurrent training, and whether the molecular response to a concurrent stimulus is modified in a concurrent-trained state.

The observations will provide evidence to assist endurance athletes undertaking concurrent training and might assist coaches and practitioners to move closer to optimising the concurrent training process. It will also provide insight into a possible mechanism regulating interference and how training variables might contribute to this process.

## **2. LITERATURE REVIEW**

## 2.1 Literature review

Accomplished performance in many professional sports necessitate the athlete to develop muscular strength and endurance simultaneously. This requirement to elicit adaptation in divergent physiology is a challenge. In particular, the ability to improve strength whilst undertaking endurance training, termed concurrent training, is thought to be troublesome. The first publication on this topic in the scientific literature was by Dr. Robert Hickson. As a young post-doctoral research fellow at Washington University, Dr. Hickson sought to impress his superior, Prof. John Holloszy, by joining him for his running training around the campus grounds. Dr. Hickson was a keen power-lifter, and in undertaking both his usual strength training alongside the endurance sessions with Prof. Holloszy, he noticed his power-lifting performance deteriorate. After discussing his observations with his mentor, the pair decided that this finding was interesting and warranted further investigation. Hence, Dr. Hickson was tasked with a research objective, once he had his own laboratory and resources to conduct a training study.

Upon obtaining a position at the University of Illinois, Dr. Hickson conducted the research study, using the back-squat to assess maximal leg strength and quantified endurance adaptation with the measurement of  $\dot{V}O_{2max}$ . Three training groups were formed of recreationally active participants, required to conduct strength (S), endurance (E), or both training modes (S + E) across a 10 wk period. As expected, the single mode training groups only improved performance in the respective outcomes. While the S + E group managed to improve endurance in line with the E group throughout the duration, maximal leg strength improvement between the S and S + E groups was comparable up until 7 wk, with a decline in the S + E group thereafter (Hickson, 1980). Hence, Dr. Hickson evidenced his anecdotal observations from his time at Washington University and termed this finding the ‘interference of strength development’. The phrase ‘interference effect’ or ‘interference phenomenon’ has been prominent within the literature in the ensuing years, as have efforts

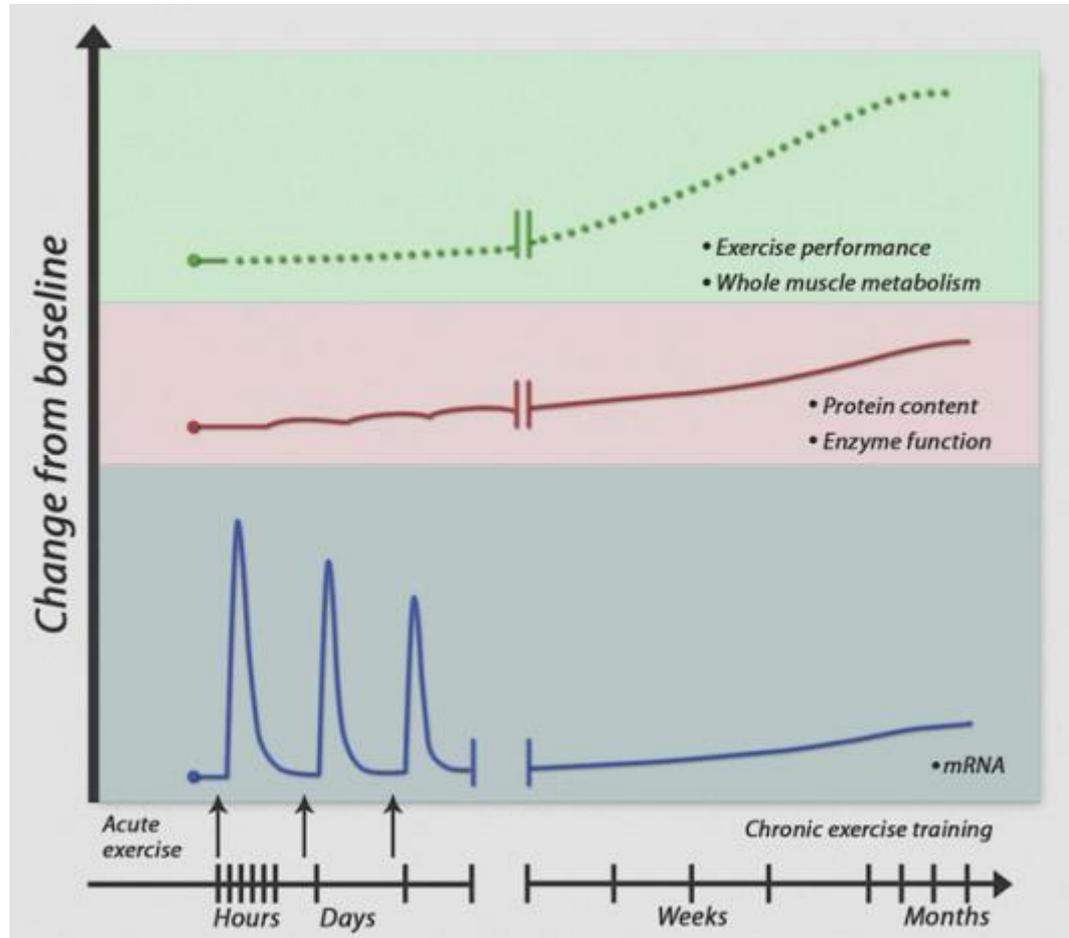
to better understand and explain this incompatibility of simultaneous strength and endurance training.

The observation of an interference effect by Dr. Hickson was pertinent, primarily owing to the vast number of athletes which employ a concurrent training paradigm. Numerous sporting populations are required to develop strength and aerobic capacity simultaneously. This is particularly true of those sports which span the middle ground of the strength-endurance continuum. These sports require athletes to possess a close balance of strength and endurance characteristics and therefore provide a distinct rationale for the individual to train concurrently. Beyond this, athletes participating in those sports which sit closer to the endurance pole of the continuum are also considered to benefit from a concurrent training paradigm, primarily owing to improvements in economy of movement (Rønnestad and Mujika, 2014). Dr. Hickson's observation of the interference phenomenon was therefore of great relevance to many athletes. This literature review will critically discuss the aforementioned body of research, with a primary focus on the interference effect presented through the practice of concurrent training.

## **2.2 Exercise stress specificity**

A single bout of contractile activity activates and/or represses signalling pathways which are specific to the stimulus, which in turn regulates transcription and translation, in addition to exercise-responsive gene expression (Egan and Zierath, 2013). These molecular mechanisms coordinate the response to exercise, leading to altered protein content and enzyme activity, which ultimately characterises the malleable nature of skeletal muscle. Hence, it is the repetition of an exercise stimulus which leads to the alteration of muscle phenotype (Perry et al., 2010). The outcome is to better prepare the muscle to provide homeostasis in light of perturbation from the exercise stress and therefore improved exercise performance in the context of the exercise challenge presented (Holloszy and

Coyle, 1984a). The temporal pattern of these events are depicted in Figure 2.1. These processes are common to all vertebrates, but the individual variation in response to exercise stress is pronounced amongst humans (Hawley et al., 2014).

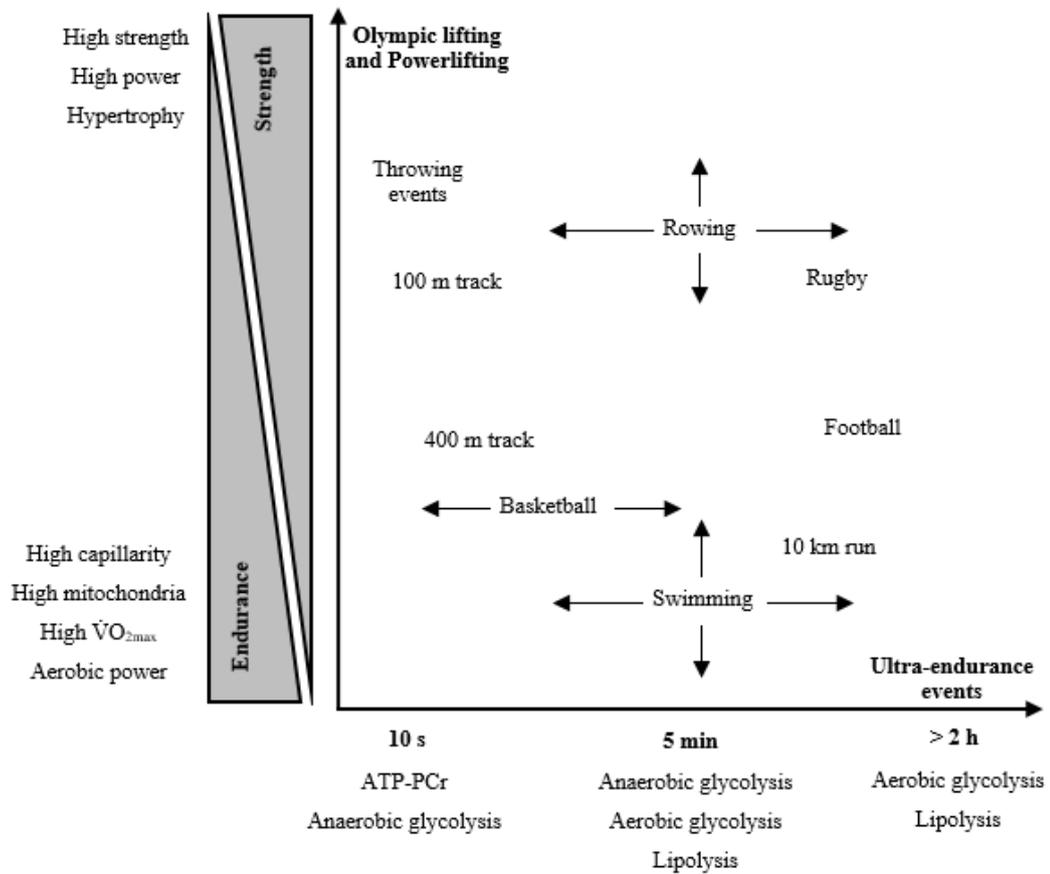


**Figure 2.1.** An illustration of the timecourse of changes from baseline in mRNA expression, protein content, and exercise performance following acute and chronic exercise training (Egan and Zierath, 2013).

The functional outcomes of repeated exercise stress are heavily influenced by acute training variables, such as exercise volume, frequency, and intensity (Fernhall and Kohrt, 1990, McCafferty and Horvath, 1977). Furthermore, exercise mode has a profound effect on the response to exercise stress. Resistance exercise is characterised by high-load, low-frequency muscle contractions, while endurance activity imposes a low-load, high-frequency demand on the muscle. In an acute context, contrasting exercise modes

differentially activate or repress signalling pathways (Coffey et al., 2006). With the repetition of an exercise stress, endurance exercise induces changes in mitochondrial protein content and the respiratory capacity of the muscle (Holloszy and Coyle, 1984a), while strength activity elicits an increase in myofibrillar protein content, driving concomitant hypertrophy of the muscle (Phillips, 2014). Functionally, the practice of a given mode of exercise, results in modifications which support improved performance in associated exercise tasks, such as preferential substrate oxidation during sub-maximal exercise for the endurance athlete and a greater contractile force output for the strength athlete (Calvo et al., 2008a, Holloszy, 1967, Phillips, 2014).

There are a number of sports and events which represent an extreme of the strength-endurance continuum i.e. Olympic lifting and ultra-endurance events. Training for adaptation within these polarised tasks is relatively well understood and linked to the processes of exercise stress specificity, described above. However, there are a multitude of sports and events which sit somewhere in-between the poles of this continuum (Figure 2.2). Training for the athletes in these sports becomes more complex, as they seek to adapt to contrasting exercise stresses. This objective to improve performance across both strength and endurance tasks, describes the very nature of concurrent training. As initially evidenced by Dr. Robert Hickson, it is the combination of training methods which poses a greater challenge for the athlete and coach, relative to single mode exercise. Beyond the rationale for improved athletic performance, this training paradigm also has relevance for improving general health and quality of life, particularly in an ageing population (Sillanpaa et al., 2009, Takeshima et al., 2004, Wood et al., 2001).



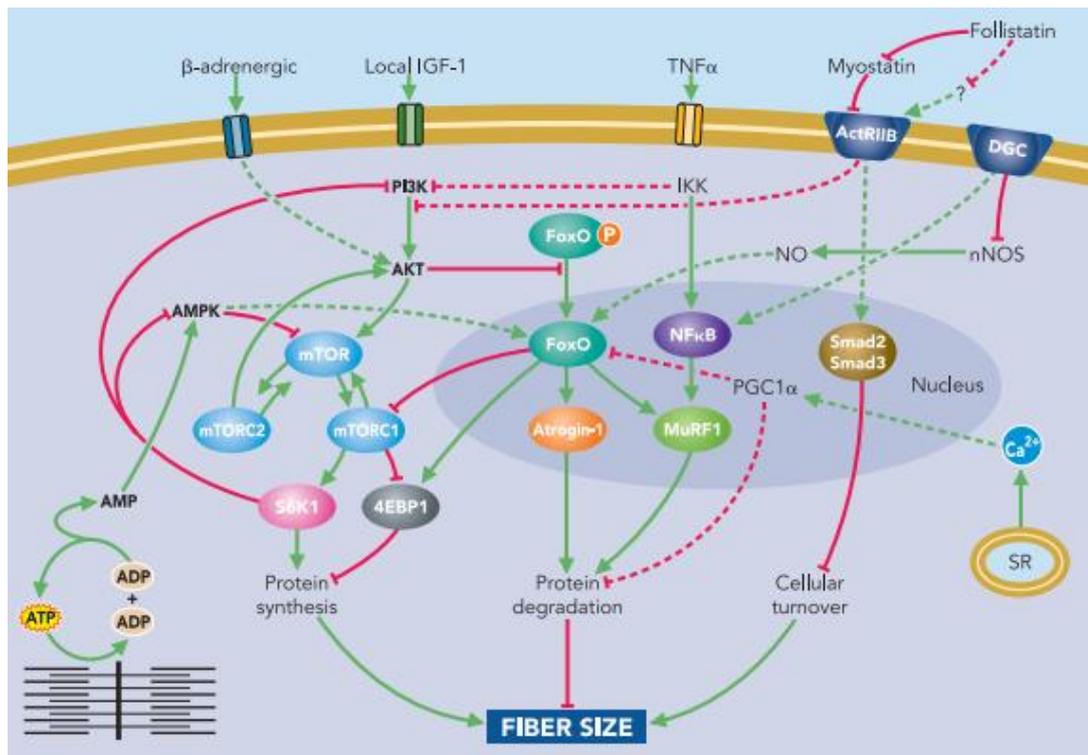
**Figure 2.2.** The strength-endurance continuum of different sporting events and disciplines, relative to exercise mode characteristics, event duration and energy metabolism, adapted from Nader (2006).

### 2.2.1 Responses to resistance training stress

A positive muscle protein balance is required to support an increase in fibre size and the maximal tension of the muscle. To facilitate this, muscle protein synthesis must exceed that of breakdown (Hawley et al., 2014). Postprandial hyper-aminoacidemia and resistance exercise stress are pre-requisites of a net gain in muscle protein (Phillips, 2014). Activating the mammalian target of rapamycin (mTOR) signalling pathway is a pertinent process of contraction-induced muscle accretion, owing to its regulatory role in muscle protein remodelling (Drummond et al., 2009, Philp et al., 2011b). Indeed, Bodine et al. (2001) demonstrated the regulatory role of the mTOR pathway *in vivo*, with activation/non-activation resulting in hypertrophy/atrophy, in addition to the blocking of hypertrophy with

the use of a genetic blockade. It should be noted however, that such data pertain to murine models and therefore fail to replicate voluntary exercise, by eliminating important neural signalling processes (Hawley et al., 2014). Such models include methods which remove synergist muscles to provide a functional overload to the muscle of interest (Bodine et al., 2001), force animals to complete an exercise protocol (Bodine et al., 2001), or use electrode stimulation apparatus to evoke muscular contractions (Baar and Esser, 1999).

The kinase mTOR exists as part of two complexes; mTOR complexes 1 (mTORC1) and 2 (mTORC2), which can be activated in a growth-factor-dependent or independent manner. mTORC1 is rapamycin-sensitive and is required for signalling to 70-kDa S6 protein kinase (p70S6K) and 4EBP1, while mTORC2 is required to signal downstream to protein kinase B (Akt) (Figure 2.3). mTOR is acknowledged to be an important regulator of cell growth, receiving signals from growth factors, energy status, and nutrients, in order to govern protein synthesis (Hay and Sonenberg, 2004). The role of mTOR in protein synthesis is regulated by p70S6K, through protein translation and ribosome biogenesis. The eukaryotic initiation factor 4E (eIF4E) - binding protein 1 (4E-BP1), and elongation factor 2 (eEF2) are implicated in the formation of translation initiation and activation of protein synthesis, with 4E-BP1 phosphorylation suppressing the inhibition of eIF4E, thereby facilitating a rate-limiting step in translation initiation. Further, the phosphorylation of p70S6K also results in the formation of the translation initiation complex, as a result of the interaction of the ribosomal protein S6 (rpS6) and the eukaryotic initiation factor 4B (eIF4B) (Sandri, 2008). p70S6K and 4E-BP1 proteins are considered the most well-defined effectors of mTOR signalling (Hawley et al., 2014).



**Figure 2.3.** An illustration of the key pathways that regulate muscle fibre size (Sandri, 2008).

The importance of p70S6K phosphorylation to the protein synthetic apparatus was highlighted by Liu et al. (2002). Authors observed the phosphorylation of Akt, 4E-BP1, and p70S6K in the *vastus lateralis* of human muscle. The findings support the role of p70S6K in the protein synthetic apparatus, with protein synthesis stimulated in an Akt-independent fashion (Liu et al., 2002). Further, a single simulated session of resistance exercise can drive an increase in the activation status of p70S6K, with the acute level of phosphorylation in different muscles correlating ( $r = 0.998$ ) with the increase in muscle mass following 6 wk of training (Baar and Esser, 1999). Such findings support the view that the acute response of signalling proteins can provide an indication of the chronic phenotypic changes which characterise muscle hypertrophy, and potentially improved strength performance (Hawley et al., 2014). It should be noted however, that the influential data from Baar and Esser (1999) relates to responses in rodents, following repeated high-force muscle contractions induced via nerve stimulation. Furthermore, the correlation identified does not support a

causal relationship between p70S6K phosphorylation and muscle hypertrophy, thereby limiting the certainty with which the acute observation of p70S6K can be used as a proxy for chronic adaptation in muscle mass. However, there are also data in human skeletal muscle to demonstrate that the regulation of Akt and its downstream targets, are associated with hypertrophy (Léger et al., 2006). Despite the purported importance of mTOR as a signalling hub, there is limited research examining the *in vivo* activation status of its key effectors in response to differing concurrent training models in human skeletal muscle.

### 2.2.2 Responses to endurance training stress

Mitochondrial biogenesis is a key training response for an endurance athlete and a process which requires the coordination of numerous cellular events. The transcriptional coactivator protein, peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), regulates the expression of mitochondrial proteins and therefore plays a prominent role in regulating mitochondrial biogenesis (Lin et al., 2005). Using an animal model, PGC-1 $\alpha$  is preferentially expressed in muscle with a high proportion of type I fibres (Lin et al., 2002). Acute endurance stimuli increases PGC-1 $\alpha$  messenger ribonucleic acid (mRNA) and protein expression in human skeletal muscle (Edgett et al., 2013, Mathai et al., 2008, Pilegaard et al., 2003, Watt et al., 2004), while high rates of expression result in an increased content of mitochondria (Lin et al., 2002).

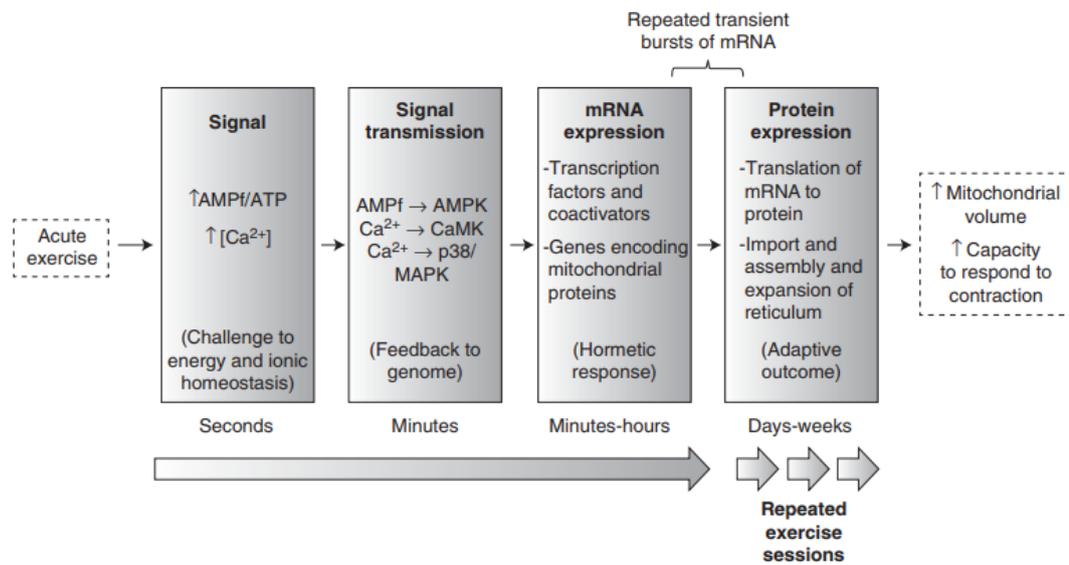
Beyond an increase in mitochondrial biogenesis, PGC-1 $\alpha$  has been linked with beneficial functional outcomes. Calvo et al. (2008b) observed PGC-1 $\alpha$  transgenic mice to better understand the potential for improved endurance performance *in vivo* with an increased expression of PGC-1 $\alpha$  in muscle, in comparison to wild-type control littermates. The PGC-1 $\alpha$  mice displayed improved performance across voluntary and forced exercise tasks, selected to represent a challenge to oxidative metabolism. Furthermore, these mice also demonstrated a greater peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) and a reduced respiratory exchange ratio (RER) in response to an incremental treadmill protocol to failure. These

results highlight the importance of PGC-1 $\alpha$  for endurance performance. However, while the authors should be commended for employing a range of exercise paradigms i.e. forced and voluntary, it should be stressed that caution still needs to be exercised when extrapolating such findings to human skeletal muscle.

Adenosine monophosphate activated protein kinase (AMPK) is a critical signalling cascade in the acute response to endurance stress, able to directly phosphorylate PGC-1 $\alpha$  (Jäger et al., 2007), and in turn, regulate mitochondrial biogenesis, along with the mitogen-activated protein kinase (p38) cascade (Hawley et al., 2014, Puigserver et al., 2001). AMPK can modulate cellular metabolism acutely, via metabolic enzyme activity (Carling and Grahame Hardie, 1989) and chronically, through the transcriptional regulation of signalling pathways (Jäger et al., 2007). The activation of this critical kinase is regulated by cellular energy status, and therefore responsive to intense exercise, nutritional signals, and oxidative stress (Howlett et al., 1998, Kahn et al., 2005). Not only is the signalling cascade sensitive to intracellular adenosine triphosphate (ATP) turnover, but Egan et al. (2010a) reported AMPK to be activated in an intensity-dependent fashion, with increased phosphorylation immediately following high (80%  $\dot{V}O_{2peak}$ ) but not low-intensity (40%  $\dot{V}O_{2peak}$ ) exercise. Ultimately, the sensitivity to cellular energy homeostasis characterises the role of AMPK and explains the observed inhibition of energy-consuming anabolic/biosynthetic processes and stimulation of catabolic processes in response to ATP depletion (Bolster et al., 2002, Kahn et al., 2005).

While the relative importance of the upstream AMPK and p38 signalling pathways is still to be elucidated, these kinases are both considered pertinent to the process of mitochondrial biogenesis (Perry and Hawley, 2018). The catabolic and regulatory subunits of AMPK act as a metabolic fuel sensor, activated in response to exercise-induced elevations in free adenosine monophosphate (AMP) (McConnell et al., 2005, Winder et al., 2000). The calcium-signalling pathway, p38 has an important role in translocating PGC-1 $\alpha$  to the nucleus, prior to increasing the activation of transcripts for mitochondrial proteins

(Wright et al., 2007). Hence, these two protein kinases are critical signals in supporting the process of mitochondrial biogenesis. In short, the process of cellular adaptation is encompassed by the aforementioned interactions. This process is more simply described by the events of sensing a signal, transmitting the signal from deoxyribonucleic acid (DNA) through to mRNA, to be translated to proteins for use at cellular locations, ultimately altering muscle phenotype (Miller et al., 2016), as depicted in Figure 2.4.



**Figure 2.4.** An illustration of the timecourse leading from acute endurance exercise, through to mitochondrial biogenesis (Perry and Hawley, 2018).

### 2.2.3 Responses to concurrent training stress

Researchers have made recent efforts to observe the acute response to concurrent exercise stimuli, with different objectives. This line of investigation has naturally resulted in combining the observation of those early post-exercise responses deemed critical to the two single modes of exercise i.e. endurance and resistance stimuli, as detailed previously (sections 2.2.1 and 2.2.2). A range of experimental outcomes have been employed to quantify the early response to exercise, including the observation of protein kinase signalling (Apró et al., 2013, Coffey et al., 2009b, Jones et al., 2015, Lundberg et al., 2012), mRNA expression (Fyfe et al., 2016b, Pugh et al., 2015, Wang et al., 2011), microRNA

activity (Fyfe et al., 2016b, Lundberg et al., 2016), and protein content (Apro et al., 2015, Carrithers et al., 2007). These outcomes have been observed from as little as 1 h (Jones et al., 2015), to as long as 6 h following a concurrent exercise stimulus (Pugh et al., 2015).

The majority of literature observing protein kinase signalling has compared a resting sample with a sample at 1 and/or 3 h post-exercise (Apro et al., 2013, Fyfe et al., 2016b, Lundberg et al., 2012, Wang et al., 2011). When reviewing study designs measuring samples at both 1 and 3 h post-exercise, Apro et al. (2013) reported greatest phosphorylation rates of Akt and AMPK signalling kinases at 1 h *vs.* 3 h, but the reverse was true of p70S6K and 4EBP1. Wang et al. (2011) observed similar timecourse outcomes for p70S6K, AMPK, and Akt phosphorylation, with comparable observations from research measuring protein kinase phosphorylation at both 1.5 and 3 h post-exercise (Apro et al., 2015). Regardless of the precise time at which the greatest post-exercise phosphorylation levels occur, significant elevations in the kinase activity of critical signalling cascades have been observed at 1 h and 3 h following concurrent exercise (Apro et al., 2015, Apro et al., 2013, Wang et al., 2011).

Despite the antagonistic nature of the interference effect theory (detailed subsequently in section 2.3), there are data displaying increased phosphorylation of protein kinases associated with both the AMPK and mTOR signalling cascades, following a concurrent exercise stimulus (Apro et al., 2015, Wang et al., 2011). Wang et al. (2011) observed increased phosphorylation of mTOR, p70S6K, Akt, and AMPK protein kinases following a concurrent exercise condition, while Apro et al. (2015) reported increased phosphorylation of Akt, mTOR, p70S6K, and AMPK. Such findings would appear suggestive of a concurrent exercise stimulus driving early responses associated with both muscle hypertrophy and mitochondrial biogenesis. Beyond this, there are data which contradict the theory of interference, when comparing a concurrent exercise condition *vs.* a resistance mode of exercise in isolation. Apro et al. (2015) reported elevated AMPK phosphorylation in the concurrent exercise condition, relative to a resistance only stimulus,

without a subsequent inhibition of mTOR activation status in the concurrent exercise condition. Further, there are data from Lundberg et al. (2012) displaying elevated mTOR and p70S6K activation status following endurance-resistance exercise, compared with resistance only exercise. Similarly, elevated mTOR phosphorylation has been observed following concurrent exercise, relative to resistance exercise (Pugh et al., 2015). Hence, the acute response to concurrent exercise can include elevated phosphorylation in the mTOR and AMPK signalling pathways, but the response is not necessarily consistent with the theory of an interference effect.

The inconsistencies in the degree of cross-talk observed in the aforementioned research might be explained by the numerous methodological differences. A great number of variables are encountered when completing concurrent exercise. This provides ample opportunity for variance amongst the methods employed in individual research studies. This is highlighted by evaluating the variance that exists between the four research investigations detailed above, with differences in 1) the intensity of the endurance exercise stimulus; 2) the training status of the participants; 3) the sequencing of the concurrent exercise stimulus; 4) the resolution of the biopsy sampling; and 5) the relief between exercise modes (Apro et al., 2015, Lundberg et al., 2012, Pugh et al., 2015, Wang et al., 2011). This variety might explain the inconsistencies in the early post-exercise signalling response within the literature. Indeed, training status (Coffey et al., 2006), endurance exercise intensity (Egan et al., 2010a), and the sequencing of concurrent stimuli (Coffey et al., 2009b) have been suggested to modify the acute response to exercise. Further research, employing exercise methods which are more likely to increase phosphorylation rates of key effectors of both mTOR and AMPK signalling cascades, would be particularly enlightening.

#### *2.2.4 Role of protein to aid recovery from training stress*

There are a multitude of recovery strategies employed to support the training process, of which protein consumption is one. Additional protein is frequently consumed by elite and recreational athletes (Petroczi and Naughton, 2008, Tsitsimpikou et al., 2011) with the intention to improve exercise recovery (Erdman et al., 2007). In a cohort of elite athletes, it is reportedly one of the most popular dietary supplement strategies, with its use more likely amongst those athletes of the highest performance level (Erdman et al., 2006). Further, it is a highly practical strategy which is accessible to all. Performing strenuous exercise bouts is associated with an increase in both muscle protein degradation and synthesis (Phillips et al., 1997). The provision of adequate nutrition is required to confer a positive protein balance following the exercise stimulus, to increase myofibrillar protein synthesis rates (Moore et al., 2009a). Therefore, the consumption of sufficient protein following the exercise stimulus is a pre-requisite for muscle anabolism and hence, muscle remodelling following mechanical stress (Levenhagen et al., 2002).

Whilst there is strong evidence for protein consumption to increase muscle protein synthesis (Cermak et al., 2012), the role of protein to support recovery following strenuous exercise stimuli is less clear. Evidence exists both for (Cockburn et al., 2008, Etheridge et al., 2008, Hoffman et al., 2010) and against the efficacy of protein supplementation to support the recovery process (Green et al., 2008, White et al., 2008, Wojcik et al., 2001). An added complication in reaching a consensus on the matter, is that much of the literature in the field suffers from limitations in experimental design. An often acknowledged limitation is that there is a lack of control concerning habitual diet (Cockburn et al., 2008, Howatson et al., 2012, White et al., 2008), resulting in the possibility of underlying discrepancies in the bioavailability of a given substrate and a likely variance in the energy balance within or between experimental groups (Pasiakos et al., 2014). This is pertinent given that essential amino acids have a stimulatory effect on muscle protein synthesis (Groen et al., 2015) and carbohydrate ingestion post-resistance exercise can act to reduce

protein breakdown (Borsheim et al., 2004). Beyond the efficacy of protein to aid recovery from single mode training stress, there is a paucity of research observing more complex exercise models, such as that offered by concurrent exercise stress. As such, it would be of interest to observe whether indices of exercise-induced muscle damage (EIMD) imposed by a concurrent exercise stimulus could be attenuated with protein supplementation.

### *2.2.5 Adaptations to strength training stress*

Strength training is undertaken to elicit an increase in the muscle fibre size and the maximal tension of the muscle, assisting the neuromuscular system to generate force and overcome resistance (Hawley et al., 2014). Scientific observation of resistance exercise was first prevalent following World War II, owing to the requirement for better understanding of the rehabilitation process (Delorme et al., 1948). Progression is essential in a successful long-term strength training programme and the manipulation of key variables is critical to this process. As such, factors including the type of resistance employed, exercise choice, order of exercise, rest interval, and the number of repetitions/sets, are vital to the goal of improving strength (Kraemer et al., 2002). Adaptations subsequent to successful strength training include improved neural recruitment patterns, increased muscle CSA, and modifications to muscle architecture, eliciting greater force generation of the muscle (Häkkinen, 1989, Kawakami et al., 1993, McCall et al., 1996, Milner-Brown et al., 1975).

Voluntary strength is not only dependent upon the quantity and quality of muscle mass, but also the extent of the activation of the muscle mass in question (Sale, 1988). The commensurate improvements in strength performance following resistance training are, in part, explained by adaptations in neural function. Improved neural recruitment patterns comprises both enhanced motor unit discharge rates (Leong et al., 1999) and synchronisation, by way of supraspinal connections between motor cortex and spinal motorneurons (Milner-Brown et al., 1975), acting to increase the recruitment of the motor unit pool (Komi et al., 1978). Electromyography (EMG) is a commonly employed method

to assess efferent neural drive. Data obtained via this method and in the context of maximal voluntary contractions, should be considered in light of the inherent constraints; including the contraction range of motion, fixing/placement of electrodes, and sampling frequency (Aagaard et al., 2002).

Interestingly, the neurological adaptations elicited by strength training are principally implicated in the early time-course improvements in strength performance. Initial improvements (<4 wk) in strength performance are attributed to enhanced maximum neural activation, with muscle hypertrophy occurring subsequently (Häkkinen, 1985, Moritani and deVries, 1979). In support of this, improvements in muscular strength have been observed in the absence of muscle hypertrophy (Costill et al., 1979). It is thought that the time-course for muscle hypertrophy is at least 6 wk, with the ability to establish exact thresholds limited by the many variables encountered i.e. training status at the onset of intervention, training programme type, and nutritional support (Phillips, 2000). An added complication in ascertaining the time-course of muscle hypertrophy, is that suggestions of increased CSA in as little as 3 wk (DeFreitas et al., 2011, Seynnes et al., 2007) could be explained in part by edematous muscle swelling (Damas et al., 2016b). It is recommended that investigations of this nature should therefore observe whole muscle CSA with a concomitant measure of muscle swelling.

An increase in the CSA of the muscle fibre is defined as muscular hypertrophy (Phillips, 2000), with the accretion of force-generating myofibrillar proteins forming a prerequisite of this process (Damas et al., 2016a, Moore et al., 2009b). The size of the muscle fibre is considered to be important to strength performance, owing to the linear relationship between CSA and the potential for maximum force generation (Finer et al., 1994). Indeed, the principal adaptations to strength training include an increase in CSA by way of an increase in myofibril size and number (Folland and Williams, 2007). Appropriate resistance training can be an effective stimulus for muscle hypertrophy (Häkkinen et al., 2003, Kanehisa et al., 2003, McCall et al., 1996), but an increase in muscle size does not

necessarily always explain improvements in strength, resulting in the precise cause of increased muscular strength remaining to be fully elucidated (Dankel et al., 2018).

Fibre type transformation is another morphological adaptation possible following strength training. The classification of fibre type is based on the basic isoforms of I (slow-twitch, oxidative), IIA (fast-twitch, oxidative and glycolytic), and IIX (fast-twitch, glycolytic), which are differentiated by their functional and metabolic characteristics. Type I fibres have properties of greater mitochondrial and capillary-fibre content (Sullivan and Pittman, 1987) and form a high percentage of fibre composition in elite endurance athletes (Costill et al., 1976b, Foster et al., 1978, Gollnick et al., 1972), while type II fibres generate comparatively greater peak power and rates of ATP resynthesis (Taylor et al., 1974, Widrick et al., 2002) and dominate fibre composition amongst well-trained strength athletes (Costill et al., 1976a, Thorstensson et al., 1977). There are data to support the fibre conversion from isoform IIX to IIA subsequent to resistance training in human models (Campos et al., 2002, Hather et al., 1991, Staron et al., 1990). These data span a training period of 8 – 20 wk and relate to the observation of modest changes in fibre type in primarily untrained cohorts. Hence, while conversion of muscle fibre composition is possible following strength training programmes, there is still uncertainty in the precise time-course of this morphological adaptation.

### *2.2.6 Adaptations to endurance training stress*

Endurance performance can be defined as an effort lasting longer than 5 min, necessitating significant and sustained energy transfer from oxidative pathways (Burnley and Jones, 2007). Endurance exercise places great demand upon the delivery of atmospheric oxygen into the mitochondrial electron transport chain and the supply of carbohydrates and lipids, enabling the aerobic resynthesis of ATP (Jones and Carter, 2000). This mode of exercise is characterised by high-frequency and low-load muscular contractions, which similar to strength training, must be repeated within sessions of

appropriate intensity, frequency, and duration, in order to elicit improvements in key parameters of aerobic function. Parameters determining aerobic capacity are motor unit recruitment by the central nervous system, the delivery of O<sub>2</sub> by the pulmonary and cardiovascular systems, and the ability of the working musculature to consume O<sub>2</sub> within the oxidative pathways (Hawley et al., 2014). Exercise economy, the lactate threshold, and the maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) characterise aerobic function and enhancing these factors can be considered to improve endurance performance (Burnley and Jones, 2007).

An increase in mitochondrial density is a key adaptation in skeletal muscle following endurance training, ultimately acting to regulate substrate metabolism by promoting greater fat oxidation and reducing carbohydrate oxidation (Holloszy and Coyle, 1984b). This adaptation in substrate metabolism during submaximal exercise will act to reduce lactate production and improve the fractional utilisation of  $\dot{V}O_{2\max}$  at a given intensity (Joyner and Coyle, 2008). The increase in the size and number of mitochondria also attenuates the increase in adenosine diphosphate (ADP), AMP, inorganic phosphate (P<sub>i</sub>) and the decrease in ATP and creatine phosphate (Dudley et al., 1987), with submaximal exercise therefore generating less disturbance to homeostasis. This improved capacity of the muscle for aerobic metabolism will ultimately increase speed across a given duration or sustainable time at a given exercise intensity i.e. fatigue resistance. Interestingly, while improvements in  $\dot{V}O_{2\max}$  can be ascribed to cardiovascular adaptations facilitating greater delivery of O<sub>2</sub> to the working musculature, such as increased stroke volume and cardiac output (Blomqvist and Saltin, 1983), improvements in submaximal exercise are explained by greater O<sub>2</sub> extraction, with a tendency for reduced blood flow following adequate training (Holloszy, 2008, Paterson et al., 1979).

Similar to the morphological adaptations in fibre type pertaining to strength training, a transfer from fast-twitch to slow-titch fibre types is possible following endurance training (Howald et al., 1985, Simoneau et al., 1985). Indeed, data from one of the pioneering studies in this field displayed an increase of 9% in slow-twitch muscle fibre

composition, occurring amongst those individuals with the lowest isoform I content prior to a 5 month training programme (Gollnick et al., 1973). Interestingly, slow-twitch fibre composition values of >95% have been observed amongst endurance athletes (Gollnick et al., 1972). Beyond this transfer to a greater slow-twitch fibre content, the relationship between IIA/IIX fibres is subject to change following endurance training, with an alteration toward the more oxidative isoform, IIA (Jansson and Kaijser, 1977). The proportion of slow-twitch muscle fibres appears important for exercise economy, at least amongst elite endurance athletes. Horowitz et al. (1994) observed a high % type I fibre group ( $72 \pm 3\%$ ) to produce a greater power output during a 1 h cycle effort equating to the same O<sub>2</sub> cost as a normal % type I fibre group ( $48 \pm 2\%$ ), demonstrating the role of fibre composition for gross efficiency in an elite cohort.

Exercise economy is acknowledged as an important parameter, able to account for a large and significant amount of the variation in endurance performance amongst athletes of a comparably high ability (Conley and Krahenbuhl, 1980). There are conflicting data regarding the ability of training to modify exercise economy. While interventional studies that recruited untrained participants have reported no change in (Overend et al., 1992) or poorer (Lake and Cavanagh, 1996) exercise economy following short-term (6-10 wk) endurance training, observational data from elite athletes suggest an improvement in exercise economy across periods of >5 yr of training (Conley et al., 1984, Jones, 1998). While this contrast in adaptation could prompt conclusions regarding outcomes being based on training status or training duration, there are also data to support improved exercise economy amongst recreationally active individuals following 6 wk training interventions (Franch et al., 1998, Jones et al., 1999). Hence, despite the importance of exercise economy to endurance performance, it remains to be elucidated as to the extent and exact exercise methods with which this pertinent parameter can be improved with training.

## 2.3 The Interference Phenomenon

Accomplished performance in many professional sports necessitate the athlete to develop muscular strength and endurance simultaneously. This requirement to elicit divergent physiology adaptation is a challenge. Hickson (1980), first reported the ‘interference effect’; a muted adaptation in strength development during a concurrent training model, in comparison to that following isolated strength training. Given the necessity for different populations to develop strength and aerobic capacity simultaneously, a significant demand has been placed upon the practice of effective concurrent training methods. It is considered an essential training paradigm for athletes competing in middle- and long-distance events in rowing and canoeing (Garcia-Pallares and Izquierdo, 2011a) and reported to improve performance amongst elite kayakers (Garcia-Pallares et al., 2009a), endurance cyclists (Rønnestad et al., 2017), and football players (Wong et al., 2010). Establishing effective training methods within a concurrent exercise paradigm requires practitioners to manipulate acute training variables, to elicit targeted adaptations for a given training cycle or intervention period. The effectiveness of the training programme therefore rests on the intricacies of manipulating exercise frequency, sequence, intensity and mode.

Early research in the field centred largely around the observation of strength, power, and endurance performance, in addition to morphological adaptation. With increasing evidence of an interference effect, researchers developed hypotheses to explain the phenomenon. Residual fatigue was initially theorised to provide a possible explanation for the interference effect, with a further speculative suggestion that biochemical processes of adaptation might prove a mechanistic reason for these observations (Hickson, 1980). Subsequent work has offered additional possibilities to explain the interference effect, such as sub-optimal intra-muscular glycogen levels post-endurance exercise acting to hinder the quality of subsequent resistance exercise (Jacobs et al., 1981), or indeed, acting to attenuate anabolic signalling (Creer et al., 2005). Further, the potential for prior endurance exercise to reduce muscular peak torque via a decline in the neural input to the muscle and peripheral

contractile mechanisms (Lepers et al., 2000), or indeed, antagonistic processes at the molecular level which inhibit the potential for strength adaptation (Fyfe et al., 2014). These purported mechanisms of interference can be grouped as “acute” and “chronic” hypotheses.

### *2.3.1 The chronic and acute hypotheses*

The processes underlying adaptation to endurance and strength stimuli differ quite considerably (as discussed in section 2.2), demonstrating the malleable nature of skeletal muscle. The chronic hypothesis simply contends that these divergent processes are antagonistic to one another, leading to sub-optimal adaptation at the skeletal muscle level, in comparison to those which would be achieved by performing either mode of training in isolation (Leveritt et al., 1999). Attempts to investigate this hypothesis have resulted in the observation of muscle fibre type transitions (Häkkinen et al., 2003, Kraemer et al., 1995, Sale et al., 1990b), muscle fibre hypertrophy (Kraemer et al., 1995, Nelson et al., 1990), motor unit recruitment (Häkkinen et al., 2003, Mikkola et al., 2012), and molecular responses to exercise (Coffey and Hawley, 2016, Lundberg et al., 2013). Evidence to support the chronic hypothesis will be discussed further in section 2.4.

The acute hypothesis proposes that interference is not the result of maladaptation from combining divergent stimuli, but rather, that the quality of the strength stimulus is compromised by the concurrent endurance stimulus (Leveritt et al., 1999). Prior endurance activity acts to reduce maximal strength performance (Lepers et al., 2000), posing an obvious threat to achieving an optimal load or volume within a maximal strength session. Hence, the theory contests that it is the residual fatigue from the endurance stimulus which is deemed to result in muted strength development over time. Attempts to test this hypothesis have been conducted most conclusively with the manipulation of inter-session recovery (Robineau et al., 2016, Sporer and Wenger, 2003b) and intra-session sequencing (Cadore et al., 2013, Pinto et al., 2015). The theory was born from the disparity in strength adaptation for the upper vs. lower body in a concurrent training design employing running

as the endurance stimulus (Craig et al., 1991). Evidence to support the acute hypothesis will be discussed further in sections 2.3.2.1 and 2.3.2.3.

### *2.3.2 Role of programme variables*

Efforts to investigate the concurrent interference effect necessitate a research design incorporating both an endurance and resistance stimulus. Beyond this commonality, research design differs considerably amongst the literature, primarily owing to the numerous programming permutations made possible with so many training variables. Indeed, the manipulation of exercise frequency, sequence, intensity, duration, and mode is prevalent within the body of work. This inconsistency in approach to exploring the interference effect limits our understanding, owing to the difficulty in comparing results across studies.

#### *2.3.2.1 Sequencing of exercise*

Regardless of the mechanism(s) underpinning the interference effect, which seem to be complex and potentially multifactorial, the role of exercise sequence has become a pertinent issue. Research investigating the interference effect has employed differing orders of endurance and strength stimuli, and it is important to understand the consequences of manipulating the acute training variable of exercise sequence. Interestingly, the seminal work of Hickson (1980) failed to report the exercise sequence of the concurrent training group, with a focus on the role of exercise sequence in the provision of an interference effect not developing until some years later. The role of intra-session exercise sequence has been investigated in both acute and chronic scenarios, via molecular signalling responses post-exercise (Coffey et al., 2009a, Coffey et al., 2009b, Jones et al., 2015) and monitoring of morphological and functional outcomes e.g. maximal strength, following a period of training (Davitt et al., 2014, McGawley and Andersson, 2013, Okamoto et al., 2007).

Data are limited concerning the acute molecular responses to alternating sequences of concurrent exercise (Coffey et al., 2009a, Coffey et al., 2009b, Jones et al., 2015). The significance of acute signalling pathways following concurrent exercise are discussed previously (section 2.2.3). Coffey et al. (2009b) first investigated the topic by alternating the sequence of strength and continuous intensity endurance stimuli. Authors noted greatest Akt phosphorylation with an endurance-resistance order, along with disparate mRNA responses suggestive of modification by exercise order. The same group followed this work with a design that replaced the endurance stimulus with 10 x 6 s maximal sprints. Order effects were observed for p70S6K phosphorylation, with preferential activation when the strength stimulus preceded the sprint intervals. Despite this, authors concluded that repeated sprints might promote interference, irrespective of exercise sequence (Coffey et al., 2009a). The most recent data suggest a similar post-exercise signalling response, irrespective of exercise order (Jones et al., 2015).

The observation of exercise sequence has been more prominent in chronic scenarios. There is support for the resistance stimulus to precede endurance exercise, with the alternate exercise sequence suggested to negatively affect the training-induced strength gains (Bell et al., 1988, Cadore et al., 2013, Pinto et al., 2015). In accordance with the acute hypothesis (section 2.3.1), Lepers et al. (2000) reported that 2 h of cycling at 65% maximal aerobic power reduced muscular peak torque by 14% in well-trained cyclists, with these outcomes ascribed to a decline in the neural input to the muscle and peripheral mechanisms. Further, endurance exercise is known to significantly deplete intra-muscular glycogen levels (Noakes et al., 1988), which is associated with a concomitant reduction in the capacity to produce optimal muscular strength (Jacobs et al., 1981), or indeed, attenuate anabolic signalling (Creer et al., 2005). Hence, the residual fatigue from the preceding endurance session could act to hinder the quality of the subsequent strength stimulus and attenuate adaptation over time.

Cadore et al. (2013) postulated that greater adaptation in lower-body dynamic 1-repetition maximum (RM) with a resistance-endurance exercise sequence might be attributed to improved neuromuscular economy, owing to improvements in strength and reduced EMG signal for a given load, with no group differences in hypertrophy. A suggested role for adjustments in neuromuscular function is also supported by Eklund et al. (2015), with increased maximal force in combination with an increase in muscle activation in the resistance-endurance training group only.

In contrast to this exercise order, Collins and Snow (1993) reported an endurance-strength sequence to preferentially improve shoulder press strength. However, given that the endurance stimulus comprised of a running modality, the outcome selection of an upper-body exercise could pose a threat to the construct validity of the work, specific to the context of the interference phenomenon. Instead, the 1-RM assessment of leg press strength was likely a more valid measure of interference, which displayed no group differences following the 7 wk training programme.

In summary, there are limited and inconsistent data pertaining to the role intra-session exercise sequence to alter molecular responses following a concurrent exercise stimulus. However, training studies addressing the topic provide more data, with a resistance-endurance exercise order throughout a training programme reported to result in increased strength (Cadore et al., 2013, Okamoto et al., 2007, Pinto et al., 2015) and hypertrophy (Pinto et al., 2014), in comparison to the exercise order of endurance preceding strength training. Other research has reported no advantage to this exercise sequence for either strength, hypertrophy, or power (Chtara et al., 2008, MacNeil et al., 2014, McGawley and Andersson, 2013). Consequently, the body of literature is equivocal and additional work is warranted to elucidate the intra-session sequence effects in a concurrent exercise paradigm. Specifically, it would be of interest to robustly examine the role of exercise sequence within the context of the concurrent training interference effect.

### 2.3.2.2 *Intensity of exercise*

The predominant research design to investigate the existence of interference has involved a comparison of a strength stimulus combined with endurance activity against the identical strength stimulus in isolation. While the strength stimulus employed in the literature does differ, it is usually of appropriate design to provide the required stimulus of the muscle, broadly in line with published recommendations (Hass et al., 2001). Given the nature of the interference effect, it is the presence of an endurance stimulus which is often manipulated in the course of investigation. Both moderate intensity constant work and high intensity interval training (HIIT) have been used to evidence an interference effect in training study scenarios (Cadore et al., 2010, Dudley and Djamil, 1985, Hickson, 1980, Kraemer et al., 1995). While the endurance stimulus employed in concurrent training research has varied from moderate intensity constant work (Hennessy and Watson, 1994, Karavirta et al., 2011, McCarthy et al., 2002) to interval training at high intensities (Coffey et al., 2009a, Dudley and Djamil, 1985), there remains a dearth of literature directly comparing endurance exercise intensity in a concurrent exercise paradigm (Fyfe et al., 2016a, Fyfe et al., 2016b). This question would be best addressed by comparison of work- and duration-matched endurance exercise stimuli.

As discussed in section 2.4.3, it is the antagonistic relationship between the mTORC1 and AMPK signalling pathways which is often cited as a potential mechanism to explain the concurrent interference effect. Indeed, several reviews have discussed the potential role, at least in part, for the up-regulation of AMPK to impair strength in a concurrent training paradigm (Baar, 2014, Coffey and Hawley, 2016, Fyfe et al., 2014). AMPK is a signalling protein measured in acute scenarios as a reference for mitochondrial responses to exercise, given its role as a regulator of cellular energy status (MacInnis and Gibala, 2016). It is sensitive to exercise intensity in humans, increasing in an intensity-dependant manner (Egan et al., 2010b, Wojtaszewski et al., 2000) and is activated to a greater extent during interval *vs.* continuous protocols, when controlled for total work done

(Combes et al., 2015). Furthermore, HIIT is able to increase the phosphorylation of AMPK in response to exercise, even amongst highly-trained endurance athletes (Clark et al., 2004).

Despite the capacity of endurance exercise intensity to differentially activate purported inhibitors of anabolic mechanisms, just one group have explored this question with an acute (Fyfe et al., 2016b) and chronic (Fyfe et al., 2016a) research design. Fyfe and colleagues investigated the effect of either HIIT or moderate intensity continuous training (MICT) endurance stimuli preceding a resistance stimulus, in recreational exercisers with some level of previous exposure to both modes of exercise. The HIIT stimulus of 10 x 2 min of cycling at 120% LT and MICT stimulus of 30 min cycling at 80% LT preceded a leg press resistance protocol in both designs, with progression of endurance stimuli employed in the chronic design, reaching a peak at 11 x 2 min at 150% LT and 33 min at 100% LT, respectively. Phosphorylation of p70S6K, rps6, and 4E-BP1 was unaffected by the manipulation of endurance exercise intensity, while mTOR phosphorylation was increased for up to 3 h post-exercise in the HIIT vs. MICT condition, with authors concluding interference of the mTORC1 signalling pathway to be unaffected by the intensity of preceding endurance stimuli (Fyfe et al., 2016b).

The aforementioned research from Fyfe et al. (2016b) is limited by the study design. As discussed in section 2.3.2.1, strength performance is affected by residual fatigue from preceding endurance activity and provides sub-optimal strength adaptation across a period of training (Eddens et al., 2018). A design which prioritised the strength stimulus would likely improve the ecological validity of research investigating an interference effect. Furthermore, the signalling response of muscle is suggested to be phenotype-specific (Coffey et al., 2006) and the recruitment of a recreationally trained cohort, ensured that the phenotype was not particularly accustomed to either of the divergent stimuli, possibly resulting in a generic molecular response (discussed further in section 2.4.4). It would therefore be of interest to examine the potential for a molecular interference effect amongst a trained cohort, with a clear rationale for training concurrently, employing a design that

facilitates an optimal anabolic response i.e. strength followed by endurance stimuli. Further, while muted AMPK phosphorylation might be expected in an endurance trained phenotype in response to an isolated endurance stimulus (Coffey et al., 2006), upregulation of AMPK activation status has been observed amongst highly trained endurance athletes, when employing an HIIT model (Clark et al., 2004). Hence, the intensity of the endurance stimulus would appear pertinent in investigations of a molecular interference affect amongst trained endurance athletes.

### *2.3.2.3 Frequency of exercise*

The investigation of frequency of concurrent exercise and the provision of interference can be classified into observations of inter-session exercise sequencing and concurrent exercise frequency. There are limited data concerning the former, whereby the frequency of divergent stimuli is manipulated within a given day, with a particular focus on sufficient recovery following individual modes of exercise (Robineau et al., 2016, Sale et al., 1990a). In contrast, more work has been conducted with a focus on the role of concurrent exercise frequency in the provision of an interference effect i.e. strength impairment when manipulating the number of concurrent exercise sessions in a given week.

Sale et al. (1990a) were first to investigate inter-session recovery in concurrent training programmes. Recreationally active students were allocated to concurrent training stimuli, with the crossover relief between divergent stimuli manipulated at 0 or 24 h. Maximal leg strength improved to a greater extent in the training group afforded recovery between endurance and strength sessions. Morphological and endurance outcomes were similar between groups, with the authors concluding that same day concurrent training might impede strength development across a 20 wk intervention (Sale et al., 1990a). Subsequent work has developed the knowledge in this field, with a design of greater temporal resolution, adding a condition with a crossover relief of 6 h (Robineau et al., 2016). The design, with arguably greater ecological validity for elite practice compared to

the earlier work of Sale et al. (1990a), supported the notion of recovery duration to influence interference. Improvements in maximal strength were lower in the condition with no recovery, in comparison to those in the groups of strength only or crossover relief of 6 and 24 h. This impairment of strength performance is true also of acute scenarios, with repetitions at 75% 1-RM influenced by recovery time following endurance stimuli. Sporer and Wenger (2003b) reported both 4 and 8 h recovery conditions to impede total leg press repetitions, in comparison to 24 h recovery or control. This concept has been embraced in the literature, as evidenced by an assessment of a periodised concurrent training cycle amongst elite kayakers. Authors reported successful adaptations across endurance and strength parameters, while making explicit that a conscious decision was made to allow 6-8 h of recovery when a strength session had to follow an endurance session (Garcia-Pallares et al., 2009b).

It has been suggested that evidence of interference during training studies might be more prominent in research prescribing a training frequency of  $\geq 3$  days per wk (Garcia-Pallares and Izquierdo, 2011a). This is supported by numerous examples of concurrent interference following research designs employing this frequency of exercise (Dudley and Djamil, 1985, Häkkinen et al., 2003, Hennessy and Watson, 1994, Hickson, 1980, Kraemer et al., 1995) and a reported dose-response in a meta-analysis from Wilson et al. (2012), representing diminished strength with an increase in the frequency of endurance exercise. Despite this, training programmes using designs with fewer than three concurrent exercise sessions per week have reported an interference effect (Chtara et al., 2008, Gergley, 2009, Izquierdo et al., 2005). While this does not refute the notion that a greater frequency of training will exacerbate the interference effect, it does question the requirement of  $\geq 3$  days per week for impairment of strength.

## **2.4 Proposed mechanisms for the interference phenomenon**

Beyond understanding the implications of manipulating acute programme variables on the acute and chronic responses to concurrent training stress, it is important to elucidate the mechanisms which underlie the observation of an interference effect. In particular, while appreciating that concurrent training can inhibit functional outcomes relative to resistance training, a fuller understanding of the mechanistic processes at play might better serve practitioners to mitigate the phenomenon. These potential mechanisms are poorly understood at present. Research from this thesis will aim to more completely resolve these questions, with the observation of early molecular responses to differing concurrent exercise stimuli.

### *2.4.1 Fatigue mechanisms*

It would seem logical that attempting to elicit adaptation at both ends of the strength-endurance continuum, would place an athlete at greater risk of encountering fatigue. From a simple standpoint, an increase in total training load would seem probable, in comparison to a single mode training paradigm. The acute hypothesis proposes that interference is not the result of maladaptation from combining divergent stimuli, but rather, that the quality of the strength stimulus is compromised by the concurrent endurance stimulus (Leveritt et al., 1999). This theory is plausible, given that prior endurance activity acts to reduce maximal strength performance (Lepers et al., 2000), posing an obvious threat to achieving an optimal load or volume within a maximal strength session. Hence, the theory contests that it is the residual fatigue from the endurance stimulus which is deemed to result in muted strength development over time. If this acute hypothesis were true, it is of great interest to elucidate the possible mechanisms responsible for the interference.

Endurance exercise is known to significantly deplete intra-muscular glycogen levels (Noakes et al., 1988), which is associated with a concomitant reduction in the capacity to produce optimal muscular strength (Jacobs et al., 1981). Although not directly

measured in the context of the interference effect, it would seem tenable that performing endurance exercise of an intensity to deplete glycogen stores, would result in a sub-optimal metabolic environment for strength training, if little rest was permitted between the two. Indeed, recommendations for concurrent training practice amongst elite performers, encourages a relief duration of 6-8 h, to facilitate glycogen repletion (Garcia-Pallares and Izquierdo, 2011b). The rationale for these recommendations were based on the observation of attenuated strength performance amongst participants accustomed to concurrent training, for at least 8 h subsequent to 36 min of either high intensity interval or moderate-intensity endurance exercise (Sporer and Wenger, 2003a). These recommendations should be treated with caution however, as Sporer and Wenger (2003a) did not include a measure of glycogen utilisation, where rates of depletion have been reported to be intensity-dependent (Gollnick et al., 1974). Further, the design incorporated relief periods of 4, 8, and 24 h following endurance stimuli. Given that strength performance did not return to control levels until the 24 h condition, it is possible that performance was attenuated far beyond the recommended 8 h relief period.

Beyond the specific purpose of the relief or recovery duration i.e. glycogen resynthesis, attempts to test the importance of recovery have been conducted most conclusively with the manipulation of inter-session recovery (Robineau et al., 2016, Sporer and Wenger, 2003b) and intra-session sequencing (Cadore et al., 2013, Pinto et al., 2015). Sale et al. (1990a) were first to investigate inter-session recovery in concurrent training programmes, with crossover relief between divergent stimuli manipulated at 0 or 24 h. Maximal leg strength improved to a greater extent in the training group afforded recovery between endurance and strength sessions. Morphological and endurance outcomes were similar between groups, with the authors concluding that same day concurrent training might impede strength development across a 20 wk intervention (Sale et al., 1990a). Subsequent work has developed the knowledge in this field, with a design of greater temporal resolution, adding a condition with a crossover relief of 6 h (Robineau et al.,

2016). Similar to previous work, the authors reported attenuated strength improvements when conducting training sessions without a relief period, even if only 6 h.

The study design of research investigating the interference effect can contain a threat to construct validity, with regards to fatigue mechanisms. The seminal work of Hickson (1980) provides an example of this. Three training groups were required to conduct either strength training  $5 \text{ d} \cdot \text{wk}^{-1}$ , endurance training  $6 \text{ d} \cdot \text{wk}^{-1}$ , or both training modes i.e. 11 sessions through the week, across a 10 wk period. As detailed previously, the strength performance of the concurrent training group tracked that of the strength training group until 7 wk, with a subsequent disparity in improvements. The greater volume performed by the concurrent training group might explain this finding, as opposed to an antagonistic relationship between the two training modes, and this design is prominent within the literature (Dudley and Djamil, 1985, Häkkinen et al., 2003, Hennessy and Watson, 1994, Kraemer et al., 1995). Such disparity in training volume between the strength and concurrent training groups could implicate non-functional overreaching (NFOR) or overtraining syndrome (OTS) as a possible mechanism for the observed decline in strength performance. Fatigue and performance decline are symptoms of both NFOR and OTS, with poor balance between training and recovery proving a potential trigger for these conditions (Meeusen et al., 2013). The work of Hickson (1980) would seem particularly susceptible to fatigue, given that the frequency of training was relatively high, even in the strength or endurance only training conditions. It has been suggested that evidence of interference during training studies might be more prominent in research prescribing a training frequency of  $\geq 3 \text{ d} \cdot \text{wk}^{-1}$  (Garcia-Pallares and Izquierdo, 2011a). This could be explained by the volume of activity resulting from performing both modes of training, with the disparity between 3 vs. 6 sessions per week proving a threshold. Despite this, training programmes using designs with fewer than three concurrent exercise sessions per week have reported an interference effect (Chtara et al., 2008, Gergley, 2009, Izquierdo et al., 2005).

### *2.4.2 Neuromuscular factors*

Strength training can elicit adaptations in neuromuscular function, as discussed in section 2.2.5. More specifically, adaptations in neural function resulting in improved motor unit recruitment, by way of enhanced discharge rates or synchronisation of the motor unit pool. Dudley and Djamil (1985) were the first authors to propose a neuromuscular rationale for the interference phenomenon, in agreement with subsequent work by Hunter et al. (1987). Dudley and Djamil (1985) presented data displaying an attenuation in strength adaptation in the high-velocity, low-force region of the force-velocity relationship in the concurrent training group, relative to resistance training in isolation. Specifically, resistance training improved maximal torque at angular velocities ranging from 0-4.19 rad · s<sup>-1</sup>, while improvements from concurrent training were limited to the range of 0-1.68 rad · s<sup>-1</sup>, despite both groups completing resistance training at an angular velocity of 4.19 rad · s<sup>-1</sup> (Dudley and Djamil, 1985).

In addition to this earlier literature, there has been more recent research by Scandinavian groups to further explore this hypothesis (Häkkinen et al., 2003, Lundberg et al., 2014, Mikkola et al., 2012). Häkkinen et al. (2003) reported concurrent training to result in attenuated rapid force production, relative to resistance training in isolation, possibly explained by a reduction in rapid voluntary neural activation. In a commendable study, containing a training intervention of 21 wk, authors reported an interference effect based on the rate of force development (RFD) during maximal explosive muscle contractions. While both the resistance only and concurrent exercise conditions resulted in comparable improvements in maximal force across the training period, an improvement in RFD and average force during the initial 500 ms of the muscle contraction, was registered solely in the resistance only condition (Häkkinen et al., 2003). The suggested importance of high velocity muscular contractions in the context of the interference phenomenon is supported by a recent meta-analysis also, with power proving to be the only parameter significantly affected by the addition of endurance stimuli (Wilson et al., 2012).

Beyond training study examples, a decline in the neural input to the muscle has been proposed to explain the reduction in muscular peak torque subsequent to a single bout of endurance activity (Lepers et al., 2000). Taken together, such findings would support the role of neuromuscular recruitment patterns to be pertinent in the potential for an interference effect in the context of the acute hypothesis. As such, it would appear necessary to include an explosive measure of strength, to enable the assessment of the impact on muscular contractions dependent upon rapid voluntary neural activation. It is important however to stress, that there are also data refuting the notion of concurrent training to impede neuromuscular recruitment patterns, in comparison to resistance training alone (McCarthy et al., 2002).

### *2.4.3 Molecular factors*

The importance of the mTORC1 and AMPK signalling pathways to the early adaptive response to strength and endurance adaptation, respectively, are discussed in Sections 2.2.1 and 2.2.2. A prominent piece of research from Atherton et al. (2005) provided evidence that these signalling cascades were sensitive to the specific mode of exercise performed. Authors reported the AMPK signalling pathway to be activated only by low-frequency stimulation, while the mTOR cascade was only activated by high-frequency stimulation of the muscle; an observation which was termed the ‘AMPK-PKB(Akt) switch’. The connotation was that endurance or resistance exercise could determine the signalling state of the muscle and potentially, the divergent training responses to these modes of exercise. An interesting further observation was that low-frequency stimulation deactivated key signalling proteins of the mTOR pathway, such as p70S6K and 4E-BP1 (Atherton et al., 2005). Despite the limitations of employing an *ex vivo* murine model to mimic endurance or resistance muscular contraction during exercise amongst humans, the work did provide a pertinent foundation, in proposing a molecular mechanism by which endurance exercise might inhibit the response of the anabolic machinery. This theory has not been observed with such clarity in humans, to date (Camera et al., 2010, Coffey et al.,

2006, Wilkinson et al., 2008). Furthermore, there are data suggesting an attenuation of mode-specific signalling responses in the trained state, encompassing an element of response plasticity (Coffey et al., 2006). Such research casts doubt on the simple regulatory model proposed by the work of Atherton et al. (2005).

Despite the non-confirmation of the AMPK-PKB(Akt) switch hypothesis within human cohorts, researchers have sought to clarify whether the observation of chronic interference might be explained mechanistically, by the acute activity of these critical protein kinases. Indeed, the activation status of and potential cross-talk between the AMPK and mTOR signalling cascades following exercise, has been observed in both animal (Atherton et al., 2005, Bolster et al., 2002) and human models (Apro et al., 2013, Coffey et al., 2009b, Fyfe et al., 2016b, Lundberg et al., 2014), to measure the putative molecular mechanisms underlying the antagonistic relationship between divergent exercise modes (Nader, 2006).

A function of AMPK is to act as an energy sensor within the cell (Hardie, 2004, Winder et al., 2006), with a remit to reduce energy-consuming processes (Jorgensen et al., 2006, Kahn et al., 2005). Protein synthesis is an energy-expensive process (Weigl, 2012) and might therefore be suppressed by AMPK (Inoki et al., 2003). Further, *in vitro* models suggest a role for AMPK to inhibit mTOR activity by a direct phosphorylation of tuberous sclerosis complex 2 (TSC2). In the context of energy conservation, AMPK activates TSC2, which subsequently inhibits the phosphorylation of key translational regulators and demonstrates an ability to directly interact with the mTOR pathway and inhibit protein synthesis (Inoki et al., 2003).

The activation status of eEF2 provides an additional consideration as a possible mechanism of interference. Endurance exercise can inhibit eEF2 activity (Rose et al., 2005), which mediates ribosomal translocation along the mRNA; the energy demanding elongation phase of translation (Browne and Proud, 2002). Specifically, eEF2 is

phosphorylated and inhibited by eEF2 kinase (eEF2K), subsequent to the activation of signalling cascades which are sensitive to an increased demand or reduced supply of energy (Browne and Proud, 2002). Owing to the regulatory role of AMPK in the activation of eEF2K, it is possible that endurance exercise might prove disruptive to the processes of protein synthesis (Horman et al., 2002).

Whilst the possible regulatory controls detailed might appear promising in more completely resolving the mechanisms of interference, it is essential that they be viewed in light of their methods of observation. Much of the research involves *in vitro* observation of animal species and such methods do not properly represent physiological conditions (Hamilton and Philp, 2013). The search for mechanistic explanation in human models has largely focussed on the activation status of AMPK and mTORC1 signalling cascades in relation to training adaptation of muscle. This line of enquiry has ultimately provided a lack of support for the theory of a molecular interference effect. Beyond data representing no differential activation of mTOR and AMPK signalling networks following the manipulation of exercise stimuli (Apro et al., 2013, Jones et al., 2015), Apro et al. (2015) reported elevated AMPK phosphorylation in the concurrent exercise condition, relative to a resistance only stimulus, without a subsequent inhibition of mTOR activation status in the concurrent exercise condition. There are data from Lundberg et al. (2012) displaying elevated mTOR and p70S6K activation status following endurance-resistance, compared with resistance only exercise. Similarly, Pugh et al. (2015) observed elevated mTOR phosphorylation in a concurrent exercise condition, relative to resistance exercise. Collectively, this *in vivo* data counter the existence of a molecular interference in human skeletal muscle, by failing to demonstrate an antagonistic relationship between mTOR and AMPK molecular outputs.

#### *2.4.4 Training status*

The majority of investigations concerning the topic of a molecular interference with concurrent training have employed untrained or recreationally active populations. The scarcity of data pertaining to trained phenotypes might be explained by the challenge of collecting muscle biopsies from competitive athletes. To this author's knowledge, there are no data examining a possible molecular interference effect amongst trained endurance athletes i.e. acute signalling responses to concurrent *vs.* resistance exercise stress. It is important to highlight, that whilst there appears no clear rationale for adding endurance activity to the training programme of a strength athlete, there is a clear rationale for the converse scenario. This is primarily owing to an improvement in economy of movement following concurrent training in an endurance phenotype, even amongst highly-trained athletes (Denadai et al., 2017). Hence, efforts to better understand and optimise adaptation following concurrent training practices amongst endurance athletes, are warranted.

The lack of data examining a molecular interference effect amongst trained endurance athletes is troublesome, given evidence that training status can act to modify the early molecular signalling responses to opposing exercise stimuli. Coffey et al. (2006) observed a degree of "response plasticity", whereby athletes failed to upregulate signalling in the pathway associated with the mode of exercise that they are trained in i.e. the AMPK signalling cascade and endurance activity. In addition, when these endurance athletes undertook a strength training bout, signalling activation status was increased in targets of both the AMPK and mTOR signalling pathways (Coffey et al., 2006). Hence, it is suggested that there is an attenuated molecular response amongst trained phenotypes i.e. endurance athlete performing endurance activity, with a generic molecular footprint response in untrained cohorts i.e. endurance athlete performing strength activity (Coffey and Hawley, 2016). This proposal would suggest untrained individuals to be a poor vehicle to explore the molecular bases of an interference effect. Importantly, the data from Coffey et al. (2006) was not representative of concurrent exercise i.e. the two exercise tasks were

isolated. Data concerning the early molecular signalling in a trained phenotype, in response to a concurrent exercise stimulus, would assist in better understanding the potential for an interference effect. As such, it would be of interest to examine whether a molecular interference is observed in response to concurrent exercise amongst trained endurance cyclists, which are strength training naïve.

A further consideration for the role of training status, is its interaction with training frequency. It is likely that trained athletes will have a greater training frequency than recreationally active individuals. Training frequency might have the potential to moderate the interference present, as discussed in section 2.3.2.3. It could therefore be argued that examining competitive athletes might increase the likelihood of observing the interference phenomenon, when employing a training study design. However, given that interference has been observed amongst untrained populations following high frequency training interventions (Bell et al., 2000, Hickson, 1980), the use of trained athletes is not a certain requirement when attempting to provoke an interference effect. Research designs able to distinguish whether the impairment of strength development across a training study are the result of training status or training frequency, would be beneficial to the literature base.

## **2.5 Summary**

There are some excellent data within the concurrent training literature, which provide great insight into the challenges of the concurrent training paradigm and the putative moderating factors. Despite this body of literature, there are pertinent questions which require further investigation. With respect to the acute hypothesis, there are limited data pertaining to the response to a bout of concurrent exercise. Few studies have sought to understand the acute impairment in capacity for a given mode of exercise following another (Jacobs et al., 1981, Lepers et al., 2000), but more so, there are no data concerning the recovery of performance parameters following an intense bout of concurrent training

activity. This will be addressed by the initial study aim; 1) to examine the recovery profile in response to a strenuous concurrent training stimulus, amongst an endurance trained cohort.

Beyond recovery from a concurrent exercise stimulus, there is much conjecture as to the potential for training variables to manipulate the muscle adaptive response to concurrent training. Specifically, there are applied recommendations regarding exercise sequence which are based on the acute response to alternating modes of exercise, as opposed to robust training outcome data (Garcia-Pallares and Izquierdo, 2011a). In addition, there are no published data investigating the role of endurance exercise intensity on the interference effect amongst trained athletes, despite the putative importance of training status for the acute response to divergent stimuli (Coffey and Hawley, 2016, Coffey et al., 2006) and the intensity-dependant regulation of AMPK phosphorylation (Egan et al., 2010b, Wojtaszewski et al., 2000). These gaps in the literature will be addressed by the subsequent study aims; 2) to assess whether intra-session concurrent exercise sequence modifies strength-based outcomes associated with the interference effect, following short-term concurrent training; 3) to examine whether acute interference is evident in an endurance trained cycling cohort, and whether endurance exercise intensity plays a role in mitigating the potential effect; 4) to investigate whether endurance exercise intensity modifies the adaptation in lower-body strength and cycling-specific endurance following concurrent training, and whether the acute molecular response to a concurrent stimulus is modified in a concurrent-trained state.

### **3. GENERAL METHODS**

### **3.1 General methods**

Many components of assessment and analysis have been repeated throughout different experimental chapters of this thesis. These consistent elements are presented in this chapter. In instances where the methods employed were specific to an individual investigation, these are presented in the respective experimental chapter.

### **3.2 Ethical approval**

Prior to data collection, all procedures were given institutional research ethics approval, in accordance with the Declaration of Helsinki (World Medical, 2013). In all experimental chapters, after being informed of the potential benefits and risks and completing a questionnaire to assess for eligibility and contraindications to the study, participants volunteered to take part in the research by providing written, informed consent. All documentation was approved by the Northumbria University Research Ethics Committee, prior to the research opportunity being advertised.

### **3.3 Participants**

For all experimental investigations, trained male endurance cyclists who were regularly competing (at least a Category 3 British Cycling licence holder or an estimated 16.1 km time trial of  $\leq 23$  min) volunteered to take part. Volunteers had to possess an endurance training history of  $>1$  yr, with no apparent contraindications to the study. All participants were non-smokers and not permitted to consume nutritional supplements in addition to the bespoke diet sourced from the tailored menu provider (Soulmatefood Ltd, Lancashire, UK).

### 3.4 Performance measures

A series of performance measures were employed to assess physical or physiological parameters of participants. Dependent upon each respective study design, these measures were assessed prior to, during, or after an intervention, with the intention to;

1. Characterise the research cohort.
2. Determine and standardise the relative training intensity prescribed.
3. Assess the effect of the experimental intervention.

#### *3.4.1 Assessment of peak oxygen uptake*

An incremental lactate threshold (LT) assessment was conducted prior to the  $\dot{V}O_{2\text{peak}}$  test, with the starting intensity selected (range: 125 – 200 W) to initiate the test below the LT, with subsequent increases in the work rate of 25 W every 4 min. This assessment was terminated with a blood lactate concentration of  $\geq 4 \text{ mmol}\cdot\text{L}^{-1}$  (range: 4 – 7 stages). After completion of the lactate threshold assessment, a 15 min period of rest was initiated. Participants then cycled at a power output of 200 W using an electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA). Power output was subsequently increased by 4 W every 10 s ( $24 \text{ W}\cdot\text{min}^{-1}$ ) until volitional exhaustion. Expired gas and heart rate were collected throughout the test, with the test being terminated when the participant was unable to maintain the workload. Expired gas data were averaged across 30 s intervals using online gas analysis software (MetaSoft\_Studio, Cortex., Leipzig, Germany), before downloading for subsequent assessment.  $\dot{V}O_{2\text{peak}}$  was calculated as the highest 30 s average collected during the maximal test. An online gas analyzer (Metalyzer 3B, Cortex., Leipzig, Germany) was used throughout the protocol to measure oxygen and carbon dioxide fractions and volume of gas in inspired and expired air. The analyzer was calibrated for oxygen and carbon dioxide fractions and gas volume (3 L syringe), as per manufacturer's instructions. During the test, participants breathed through a low dead space (70 mL) mouth piece, low resistance turbine, while inspired and expired gas was sampled

continuously at 50 Hz. Routine quality assurance records demonstrated a laboratory coefficient of variation for  $\dot{V}O_2$  data of 1.8% in the range of 2.05–3.94 L·min<sup>-1</sup>.

### 3.4.2 Counter-movement jump

Counter-movement jump (CMJ) performance was assessed using the OptoJump system (OptoJump, Microgate S.r.l., Bolzano, Italy), with three maximal efforts performed on each testing occasion, each separated by 60 s rest. Participants were instructed to place their hands on their hips, descend rapidly to ~90° knee joint angle, and then jump as high as possible. Standardised verbal encouragement was provided for each effort and the peak value generated across the three repetitions was used for data analysis. The intra-individual reliability of this measure returned a coefficient of variation of 0.9%.

### 3.4.3 Maximal strength testing

Maximal strength was predicted from participants' 5-RM performance in the relevant exercise, using the following; *Eq. (1) 1-RM = 100 · rep wt / (48.8 + 53.8 · exp [-0.075 · reps])* (Wathan, 1994), which previously reported good agreement with 1-RM performance in individuals naïve to strength training (LeSuer et al., 1997). It was deemed that a 5-RM assessment would be a safer method of assessment for the participants, given their lack of strength training experience. The three strength exercises used within this thesis were the back-squat, split-squat, and calf-raise. The squat technique is reported to provide a potent stimulus of the *vastus lateralis*, comparative to that of alternate lower-body strength exercises (Ebben et al., 2009). Further, these three exercises are reported to improve parameters of strength, jump height, and muscle CSA amongst trained cyclists (Rønnestad et al., 2010, Rønnestad et al., 2017). The assessments were conducted in line with standardised procedures (Ebben et al., 2009, Rønnestad et al., 2012) and supervised by a qualified strength and conditioning coach. A standardised warm-up, lasting approximately 10 min, was completed before the first exercise. This warm-up consisted of shuttle runs,

inch worms, lunges, walking Romanian deadlifts, squat rotations, glute bridges, and planks, followed by three sets of back-squat of increasing load (40, 75, and 85% of predicted 1-RM) and decreasing number of repetitions (10, 8, and 6, respectively). The first 5-RM attempt was lifted with a load ~5% below the predicted 5-RM. After each successful attempt, the load was increased by 2-5%, with one re-attempt permitted per load. The rest period between each attempt was standardised at 3 min and the last successful set was accepted as the 5-RM score. If more than one exercise was being assessed, a back-squat, split-squat, calf-raise order was followed.

### **3.5 Acute exercise stimulus**

An acute exercise stimulus was employed in Chapters 6 and 7, in order to assess early molecular responses to exercise stress. In these instances, the exercise stimulus comprised of either resistance exercise alone, or this resistance stimulus followed by endurance exercise of either moderate or high intensity. Owing to the mechanistic nature of the observations from these visits, strict visit preparation routines were followed (detailed subsequently). Standardised verbal encouragement was provided to motivate participants through these exercise protocols.

#### *3.5.1 Exercise and dietary control*

For 24 h prior to an experimental trial, participants refrained from structured exercise and consumed a standardised diet. Dietary intake was controlled for 24 h prior to arrival at the laboratory, through to completion of the visit. Daily dietary intake was standardised (6 g·kg<sup>-1</sup>·d<sup>-1</sup> carbohydrate, 1.3 g·kg<sup>-1</sup>·d<sup>-1</sup> protein, 0.98 g·kg<sup>-1</sup>·d<sup>-1</sup> fat), with the evening meal (7:00 PM) and breakfast meal (6:00 AM) prior to the visit standardised at 3 g·kg<sup>-1</sup>·d<sup>-1</sup> carbohydrate, 0.5 g·kg<sup>-1</sup>·d<sup>-1</sup> protein, 0.3 g·kg<sup>-1</sup>·d<sup>-1</sup> fat and 1 g·kg<sup>-1</sup>·d<sup>-1</sup> carbohydrate, 0.1 g·kg<sup>-1</sup>·d<sup>-1</sup> protein, <0.01 g·kg<sup>-1</sup>·d<sup>-1</sup> fat, respectively. The diet was sourced

from a tailored menu provider (Soulmatefood Ltd., Lancashire, UK) and designed in line with American College of Sports Medicine (ACSM) recommendations (Thomas et al., 2016). Food was delivered to participants' home address and labelled such that four meals were provided daily, to be consumed at specified times, ensuring that food distribution throughout the day was standardised. Food content was analysed using dietary analysis software (Nutritics v4.108, Nutritics Ltd., Co. Dublin, Ireland), in order to confirm content against request.

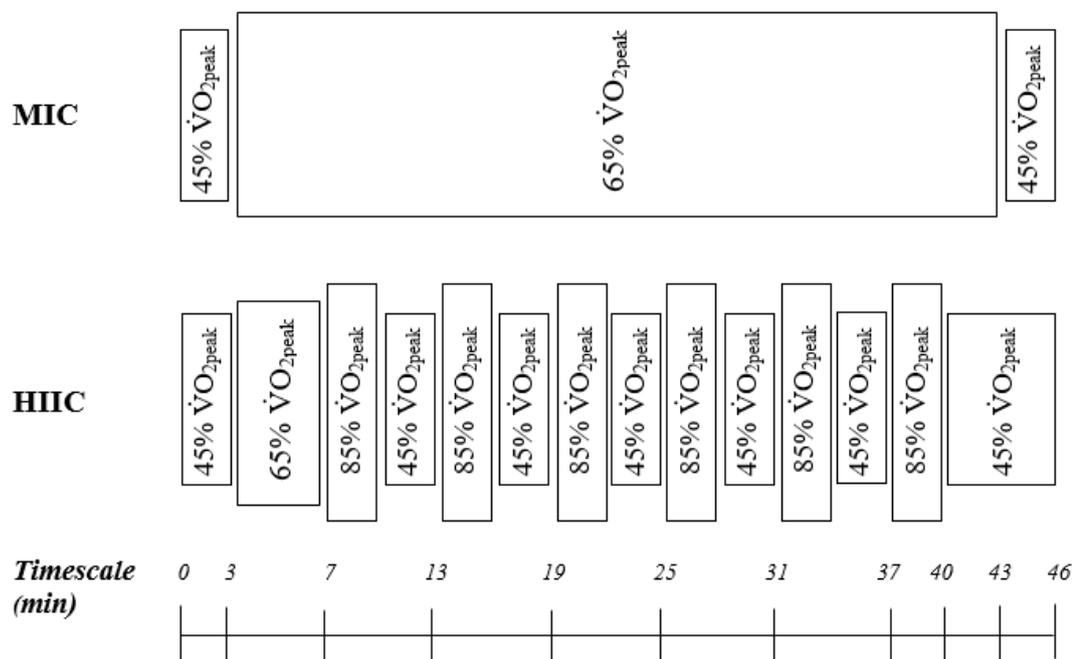
### *3.5.2 Resistance exercise stimulus*

After performing a standardised 10 min dynamic warm-up consisting of shuttle runs, inch worms, lunges, walking Romanian deadlifts, squat rotations, glute bridges, and planks, participants completed two warm-up sets of the back-squat (10 and 8 repetitions at 40 and 60% of predicted 1-RM, respectively). Consistent with the maximal strength testing, RES was conducted in line with standardised procedures and supervised by a qualified strength coach. Participants completed 6 x 8 repetitions at 80% of predicted 1-RM, with the rest period between each set standardised at 3 min. This session composition is in agreement with the ACSM position stand (2009) and similar session composition has been reported to upregulate protein phosphorylation targets of the mTOR pathway (Coffey et al., 2009b, Parr et al., 2014). If prescribed, participants commenced the endurance exercise stimulus (described subsequently) within 5 min of completing RES.

### *3.5.3 Endurance exercise stimulus*

Participants completed either moderate intensity cycling (MIC) or work- and duration-matched high intensity interval cycling (HIIC), dependent upon randomisation. MIC entailed constant load cycling at 65%  $\dot{V}O_{2peak}$ , while HIIC required participants to perform 3 min intervals of 85 and 45%  $\dot{V}O_{2peak}$ , using an electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA). The MIC condition of 65%  $\dot{V}O_{2peak}$  provided a standard prescription of endurance exercise intensity of constant duration (Apro

et al., 2013, Coffey et al., 2009b, Jones et al., 2015). The exercise of 3 min intervals of 85 and 45%  $\dot{V}O_{2peak}$  provided a matched high intensity intervention (Seiler and Tønnessen, 2009b). Further, similar stimuli have previously been used to investigate biochemical responses to endurance activity (Bartlett et al., 2012). Both protocols contained a warm-up and cool down and are presented in Figure 3.1. Heart rate was recorded throughout each trial (Polar A300 transmitter, Polar Electro Ltd., Kempele, Finland), while visual feedback of time elapsed, power output, and pedal cadence were made available to participants.

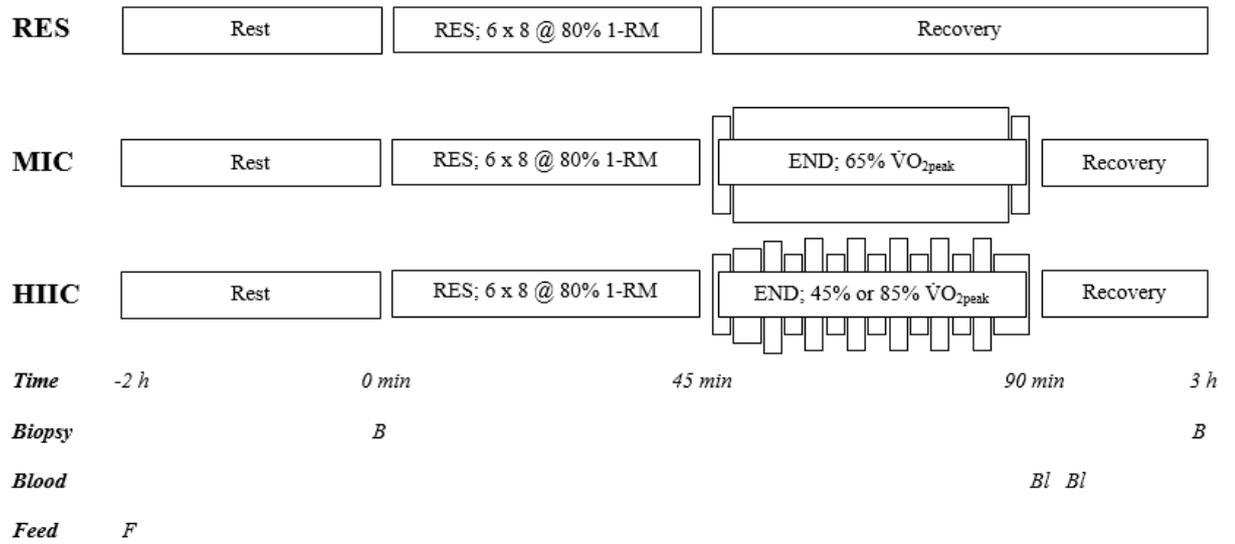


**Figure 3.1.** Schematic of the work and duration-matched MIC and HIIC protocols, forming the endurance exercise element of the acute exercise stimulus. MIC = moderate intensity cycling; HIIC = high intensity interval cycling.

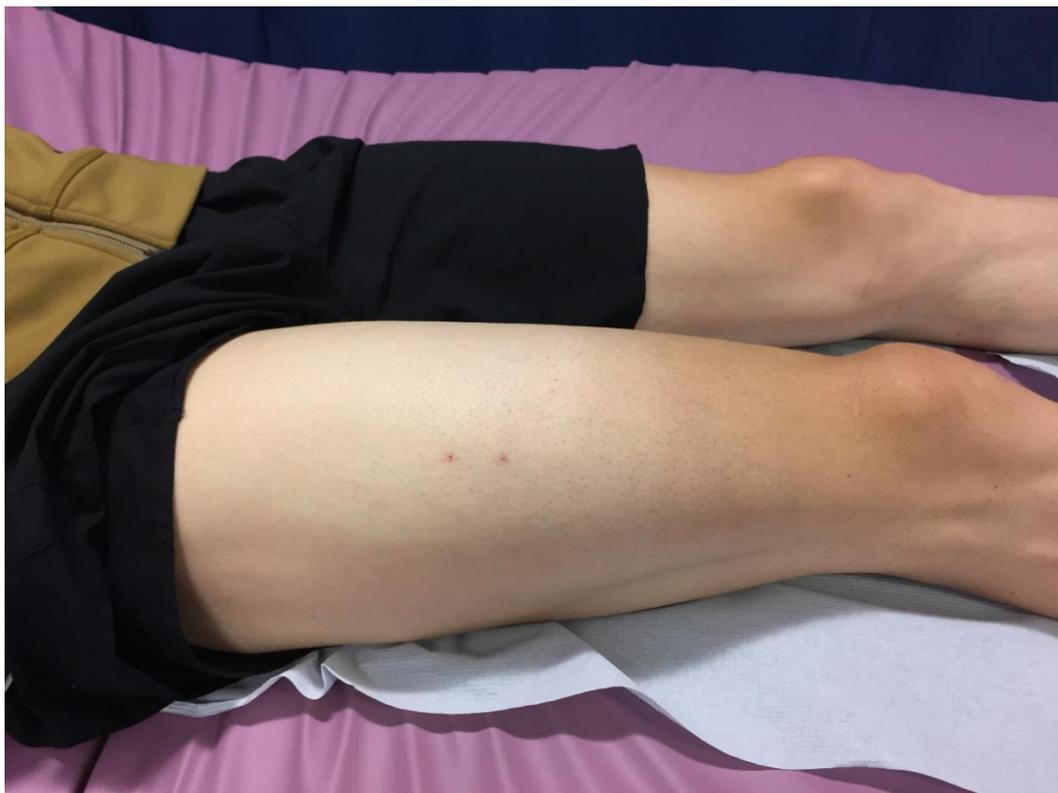
### 3.5.4 Muscle sampling

Upon arrival at the laboratory (~7:30 AM), participants were screened for contraindications to the muscle biopsy procedure, before resting in a supine position (10 min). All sessions commenced at the same time of day ( $\pm 30$  min), to minimise the effects of diurnal variation in molecular responses to the exercise stimuli (Atkinson and Reilly,

1996). Muscle samples were collected from the middle portion on the lateral aspect of the *vastus lateralis* muscle, using the micro-muscle biopsy technique. The site was disinfected with Betadine (Purdue Pharma., Connecticut, USA) and samples were obtained under local anaesthesia, with 2 ml of 1% Lidocaine Hydrochloride (Hameln Pharmaceuticals., Gloucester, UK) injected into the subcutaneous tissue of the biopsy site. After confirming that the anaesthetic had taken effect (~5 min), a 14 gauge co-axial needle was inserted ~2 cm into the muscle (beyond the subcutaneous tissue). A disposable biopsy instrument (TSK Stericut Biopsy Needle 14 Gauge, TSK Laboratories., Tochigi, Japan) was subsequently inserted through the co-axial and discharged. A single muscle sample was collected (~ 10-20 mg) and the tissue was immediately frozen in liquid nitrogen, before being stored at -80°C until subsequent analysis. If required, a second pass was completed, with the biopsy instrument rotated 180° inside the co-axial needle. Biopsies were obtained immediately prior to RES and 3 h after completion of RES (Figure 3.2), with participants resting in a waiting room for the interval between the end of exercise and the final biopsy. All within-trial biopsies were sampled from the same leg (Figure 3.3), while between-trial biopsies were sampled from alternate legs. The second sample of the visit was collected ~3 cm proximal to the greater trochanter, in respect to the first sample, in a bid to avoid a downstream haematoma, which has been observed with the more invasive Bergstrom muscle biopsy technique (Van Thienen et al., 2014).



**Figure 3.2.** Schematic of the single exercise stimuli. RES = resistance exercise; END = endurance exercise.



**Figure 3.3.** The rest and 3 h post-resistance exercise biopsy locations.

## 3.6 Biochemical analyses

Biochemical analyses were conducted to assess metabolic and signalling markers, in order to quantify mechanistic information in relation to the biological response or adaptation to the exercise stimuli.

### 3.6.1 Blood lactate analysis

A 20  $\mu\text{L}$  capillary blood sample was collected from the fingertip into a capillary tube and analysed immediately using an automated blood lactate analyser (Biosen C-Line, EKF Diagnostics., Cardiff, UK). Routine quality assurance records demonstrated a laboratory coefficient of variation for blood lactate measurement of 0.27%, in the range of 2–18  $\text{mmol}\cdot\text{L}^{-1}$ .

### 3.6.2 Muscle analysis

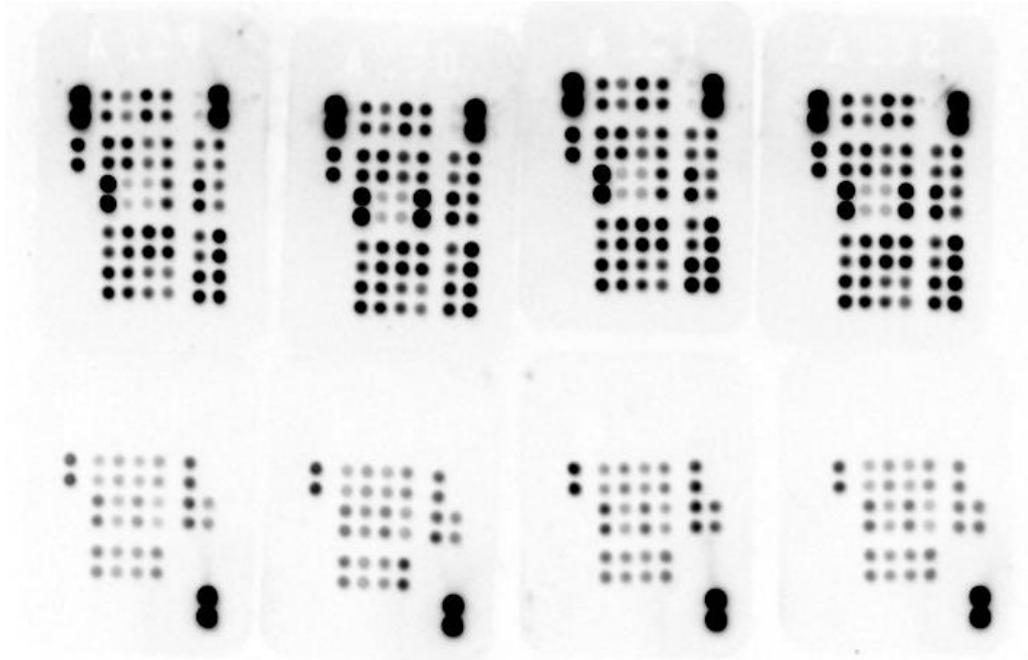
All muscle samples were analysed using a human phospho-kinase array (Proteome Profiler; no. ARY003B, R&D Systems., Minneapolis, USA), as per the manufacturer's instructions. Briefly, approximately 10 mg of muscle tissue was homogenised in ice-cold lysis buffer. Samples were rotated end-over-end for 30 min at 4°C and centrifuged at 13,000 g for 6 min, and the supernatant subsequently collected. Protein concentration was determined using a total protein assay (Pierce BCA Protein Assay; no. 23225, Thermo Scientific., Rockford, USA), with a starting range of 400  $\mu\text{g}$  per array. The nitrocellulose membranes with spotted capture and control antibodies, were blocked with array buffer 1 for 1 h at room temperature on a rocking platform shaker. Cell lysates were then diluted to a final volume of 2 mL with array buffer 1 and membranes rocked in solution overnight at 4°C. Membranes were subsequently washed to remove unbound proteins and incubated for 2 h at room temperature with the respective antibody solution (diluted detection antibody cocktail A or B). After washing, membranes were incubated for 30 min in a diluted streptavidin horseradish-peroxidase solution and protected from light, while being rocked at

room temperature. After being washed again, chemiluminescent detection reagents were spread evenly onto the membranes and incubated for 1 min, before removing excess solution and measuring the amount of bound phosphorylated protein with a 15 min exposure, using a Syngene G:Box XR5 imaging system with GeneSys analysis software (Syngene., Cambridge, UK). All sample time points for each participant were run in parallel. Figure 3.4 depicts the membrane placement ahead of imaging.



**Figure 3.4.** A depiction of the nitrocellulose membrane placement for the imaging process.

After imaging, the average signal produced at the duplicate capture spots was quantified for each phosphorylated kinase protein with the ImageJ application (National Institute of Health, USA). In brief, the region of interest on each membrane was measured with the same frame, producing a pixel density for each spot. An inverted value was calculated per protein, with net values calculated by subtracting the inverted background. Finally, a protein ratio value was calculated by taking a ratio of the net value over the reference control, allowing for the relative quantification of phosphorylation between experimental conditions. Figure 3.5 depicts the image created by the analysis software.



**Figure 3.5.** An illustrative JPEG generated by the GeneSys imaging software.

### **3.7 Statistical analyses**

Owing to the variation in experimental design employed throughout this thesis, the specific statistical analysis techniques are inconsistent between experimental chapters. As such, data are presented in detail in the respective experimental chapters of this thesis. However, inferential statistics were employed in all experimental chapters, with the use of significance testing. This process enabled the generation of a conclusion regarding an effect in the population, based on data from a sample (Hopkins and Batterham, 2016). Specifically, whether the effect is significant or non-significant i.e. the probability of an observed difference being due to chance.

**4. THE ACUTE RESPONSE TO A  
MUSCLE-DAMAGING  
CONCURRENT TRAINING  
STIMULUS AND EFFICACY OF  
PROTEIN SUPPLEMENTATION TO  
AID RECOVERY**

## 4.1 Abstract

This study investigated the profile of recovery within an EIMD context, following muscle-damaging concurrent exercise, and whether nutritional supplementation strategies would act to better support this process. Moreover, both mechanistic and applied parameters were employed to ascertain the effect of performing a challenging concurrent exercise stimulus. Twenty-four well-trained male cyclists were randomised to three independent groups receiving 20 g protein hydrolysate, iso-caloric carbohydrate or low-calorific placebo supplementation, per serve. Supplement serves were provided twice daily, from the onset of the muscle-damaging exercise, for a total of four days and in addition to a controlled diet (6 g·kg<sup>-1</sup>·d<sup>-1</sup> carbohydrate, 1.2 g·kg<sup>-1</sup>·d<sup>-1</sup> protein, remainder from fat). Following the concurrent exercise session at time-point 0 h; a simulated high-intensity road cycling trial and 100 drop-jumps, recovery of outcome measures was assessed at 24, 48 and 72 h. The concurrent exercise protocol was deemed to have caused EIMD, owing to time effects ( $p < 0.001$ ), confirming decrements in maximal voluntary contraction (peaking at  $15 \pm 10\%$ ) and countermovement jump performance (peaking at  $8 \pm 7\%$ ), along with increased muscle soreness, creatine kinase and C-reactive protein concentrations. No group or interaction effects ( $p > 0.05$ ) were observed for any of the outcome measures. The present results indicate muted performance decrements in comparison to those from single mode EIMD literature. It was postulated that this finding might have been influenced by the metabolic challenge of the cycling protocol, performed prior to the resistance exercise stimulus. Further, protein supplementation does not attenuate any of the indirect indices of EIMD imposed by concurrent exercise, when employing great rigour around the provision of a quality habitual diet and the provision of appropriate supplemental controls.

## 4.2 Introduction

Performing a novel or unaccustomed bout of exercise can result in EIMD that can negatively affect the ability to meet a high intensity demand during subsequent exercise. Specifically, a temporary reduction in maximal force production, increased muscle soreness, muscle swelling, passive tension, and the appearance of intramuscular proteins in the blood (Howatson and van Someren, 2008) are observed following the exercise. Of the indirect indices utilised in research, those that measure the functional capacity of the muscle, such as force production, appear to be the most meaningful index of EIMD (Warren et al., 1999). The presence of EIMD and hence, a decline in the force generating capacity of the muscle, poses obvious implications for athletic populations.

There is scant research concerning recovery from concurrent exercise. Concurrent exercise involves the simultaneous incorporation of both endurance and resistance exercise, (Fyfe et al., 2014) and is a prevalent training method for elite and recreational athletes that are aiming to elicit divergent physiological adaptations in parallel. Previous work examining recovery from EIMD has used resistance (Harrison and Gaffney, 2004, Miyama and Nosaka, 2004) or endurance (Bell et al., 2014, Betts et al., 2009) stimuli in isolation. Hypotheses exist for both mechanical and metabolic processes to determine the initial event of muscle fibre injury (Armstrong et al., 1991). Eccentric muscle action protocols have historically proved an effective means to impose damage, with sarcomere length inhomogeneity leading to mechanical disruption of the cell membrane (Morgan, 1990, Proske and Morgan, 2001). Further, exercise of a prolonged nature can result in degenerative changes to the muscle fibre, which lead to fibre necrosis, evidenced by the accumulation of macrophages and phagocytes (Armstrong, 1986). Hence, a concurrent exercise stimulus, incorporating both eccentric muscle actions through resistance exercise and prolonged endurance activity incorporating high intensity efforts, could prove an effective method to elicit muscle damage. Beyond the rationale of investigating concurrent exercise in the context of the hypotheses of muscle fibre injury, it is of interest to observe

the profile of recovery from the combination of demanding resistance and endurance stimuli.

Performing strenuous exercise bouts that precipitate EIMD is associated with an increase in both muscle protein degradation and synthesis (Phillips et al., 1997). The provision of adequate nutrition is required to confer a positive protein balance following the exercise stimulus to increase myofibrillar protein synthesis rates (Moore et al., 2009a). Therefore, the consumption of sufficient protein following the exercise stimulus is a prerequisite for muscle anabolism and hence, muscle remodelling following mechanical stress (Levenhagen et al., 2002). Further, this critical adaptive process of skeletal muscle might facilitate an attenuation in the indirect markers of EIMD following protein consumption (Saunders, 2007). Willoughby et al. (2003) reported an up-regulation of the ubiquitin-proteolytic pathway 48 h following an eccentric exercise bout; indicative of conditions that result in a reduced myofibrillar protein content (Hobler et al., 1998). Hence, efforts to alter protein metabolism and support myofibrillar protein synthesis as soon as possible following EIMD might facilitate recovery.

Additional protein is frequently consumed by elite and recreational athletes (Petroczi and Naughton, 2008, Tsitsimpikou et al., 2011) with the intention to improve exercise recovery (Erdman et al., 2007). The evidence is equivocal, with research suggesting efficacy (Cockburn et al., 2008, Etheridge et al., 2008, Hoffman et al., 2010) and a lack of benefits (Green et al., 2008, White et al., 2008, Wojcik et al., 2001) for protein or coingested-protein supplementation to attenuate indices of EIMD. Fractional synthesis rates and fractional net balance are still elevated at 48 h following resistance exercise (Phillips et al., 1997). Hence, muscle remodelling can be stressed for at least 48 hours following a resistance exercise stimulus. Therefore, efforts to investigate the efficacy of a protein supplement following EIMD might benefit from providing additional protein across this timeframe of remodelling, increasing bioavailability compared to single bolus supplementation models. Further, it is suggested that study designs where participants are

in negative nitrogen and/or energy balance will present the greatest potential for ergogenic effects associated with protein supplementation (Pasiakos et al., 2014). It is therefore critical that research in this field is conducted with rigorous dietary control, whereby an intervention is assessed against appropriate controls and in addition to a standardised habitual diet.

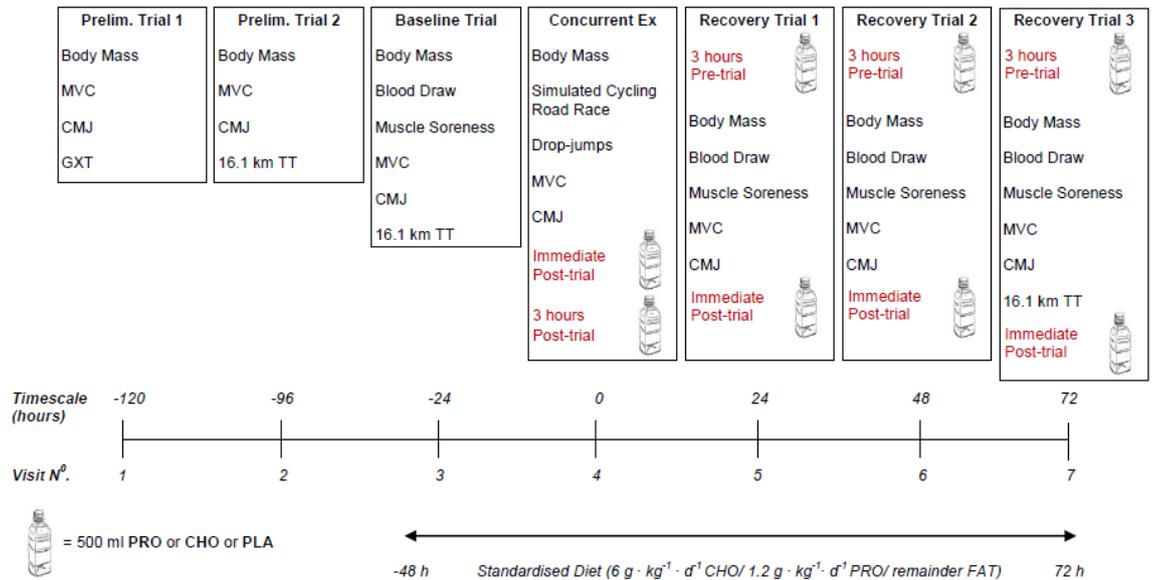
It is therefore of interest to observe whether indices of EIMD imposed by a concurrent exercise stimulus can be attenuated with protein supplementation. Beyond this, increasing our understanding of recovery from EIMD within such a pertinent training paradigm would be of importance, particularly so, given recent requests for greater specificity and context in post-exercise recovery recommendations (Minett and Costello, 2015). Consequently, the aim of the study was to investigate the effect of protein supplementation on recovery following muscle-damaging exercise, induced with a concurrent exercise paradigm, in the context of a quality habitual diet which meets current guidelines (Thomas et al., 2016).

## **4.3 Methods**

### *4.3.1 Design*

The study utilised an independent group design that was double-blind, randomised and placebo-controlled (Figure 4.1). Following two preliminary trials for familiarisation to performance tests and collection of demographic characteristics, participants attended the laboratory on five consecutive days at the same time of day ( $\pm 1$  h) to minimise the effects of diurnal variation. At each visit, participants were deemed fit for testing if they could confirm that they were free from concomitant medications/vitamins and had refrained from external exercise, caffeine and alcohol since their last visit to the laboratory (24 h). Participants also refrained from consumption of nutritional supplements for the duration of

the study period. In order, baseline data were collected for body mass (Seca 704 r, Seca., Hamburg, Germany), indices of muscle damage (creatine kinase)/inflammation (C-reactive protein), perceived muscle soreness, isometric MVC of the dominant knee extensor, maximal CMJ height and cycling TT performance.



**Figure 4.1.** Study schematic. MVC = maximal voluntary contraction; CMJ = counter-movement jump; GXT = graded exercise test; PRO = protein; CHO = carbohydrate; PLA = placebo.

Participants were then randomly assigned in a block fashion, using computer software, to one of three supplement groups; (1) whey protein hydrolysate, (2) iso-caloric carbohydrate, (3) low-calorific placebo. Supplements were consumed twice daily, for a period of four days, commencing at the end of the concurrent exercise visit, following the CMJ (Figure 4.1). Non-supplemental dietary intake was standardised (6 g·kg<sup>-1</sup>·d<sup>-1</sup> carbohydrate, 1.2 g·kg<sup>-1</sup>·d<sup>-1</sup> protein, remainder from fat) and controlled during the trial (-48 to 72 h). Each group completed the concurrent exercise session at time-point 0 h; a simulated high intensity road cycling trial and 100 drop-jumps. Recovery from the concurrent exercise bout was assessed by repeating baseline measures at 24, 48 and 72 h. Exceptions were TT performance (repeated at 72 h only) and MVC/CMJ (also assessed at 0 h). TT performance has previously been reported to be impaired at 48 h following

damaging exercise (Burt and Twist, 2011a). However, TT performance was repeated only at 72 h, in a bid to improve ecological validity, by better mimicking the amount of time that would be allowed for recovery from a heavy training stimulus prior to a competitive effort.

### *4.3.2 Participants*

Twenty-four male, well-trained endurance cyclists (age  $27 \pm 4$  years; height  $177.5 \pm 7.9$  cm; mass  $73.7 \pm 8.9$  kg;  $\dot{V}O_{2\text{peak}}$   $61.2 \pm 5.8$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) volunteered to take part in the study. Prior to data collection, the research methods were registered as a clinical trial (ClinicalTrials.gov, [www.clinicaltrials.gov](http://www.clinicaltrials.gov), NCT02458599). All study procedures were conducted in a laboratory accredited by the British Association of Sport and Exercise Sciences. Additional information relating to the ethical approval of the study and its participants is presented in the General Methods section, 3.2 and 3.3, respectively.

### *4.3.3 Procedures*

#### *4.3.3.1 Nutritional supplement*

The nutritional contents of the supplements are listed in Table 4.1. Participants ingested 500 ml of either HYDRO.365 Berry (Arla Foods Ingredients Group P/S., Viby, Denmark), Powerade ION4 Berry and Tropical (Coca-Cola Enterprises., Middlesex, UK) or Powerade Zero Berry and Tropical (Coca-Cola Enterprises., Middlesex, UK) on each supplement occasion. The supplement choice was such that the PRO condition provided 20 g of whey protein hydrolysate as an experimental intervention, with the CHO condition providing an iso-caloric control and the PLA condition a low-calorific placebo. This approach would help to establish whether potential ergogenic effects of protein supplementation were owing to protein or increased energy consumption. A total of eight supplemental beverages were consumed and these occurred at 3 h preceding, directly following, or 3 h following a laboratory visit during visits 4-7 (Figure 4.1). Each serving was in a ready-to-drink format, which was blinded to both participant and investigator

(Figure 4.2), and dispensed by a scientist that was independent to the study. Beverages were dispensed on a daily basis, with one consumed immediately following the CMJ (visits 4, 5 and 6) or 16.1 km TT protocols (visit 7) and the other(s) at a time detailed in the daily food plan. In order to test the blinding process at study completion, the dispensing scientist asked participants which supplemental condition they perceived to have been assigned to. The blind was not broken until all statistical analyses were complete.

**Table 4.1.** Nutritional content of the supplements.

	<b>PRO</b>	<b>CHO</b>	<b>PLA</b>
Energy (kcal)	90	90	5
Protein (g)	20	Nil	Nil
Carbohydrate (g)	1.85	20.5	Nil
Fat (g)	0.05	Nil	Nil

**Note:** Quantities are per 500 ml serve. PRO = protein; CHO = carbohydrate; PLA = placebo



**Figure 4.2.** A blinded single serving of supplemental beverage.

#### *4.3.3.2 Dietary control*

Non-supplemental dietary intake was standardised (6 g·kg<sup>-1</sup>·d<sup>-1</sup> carbohydrate, 1.2 g·kg<sup>-1</sup>·d<sup>-1</sup> protein, remainder from fat) and controlled during the trial (-48 to 72 h). The diet was sourced from a tailored menu provider (Soulmatefood Ltd, Lancashire, UK) and designed in line with ACSM recommendations (Thomas et al., 2016). In detail, carbohydrate and protein were prescribed relative to body mass, before selecting a range (20 - 27%) for the contribution of energy from fat, which was within the range recommended by the ACSM guidelines; 20 - 35%. These criteria resulted in categories for daily total kcal, which were a result of body mass; 2,500 kcal = 63.0 - 69.4 kg/2,750 kcal = 69.5 - 76.4 kg/3,000 kcal = 76.5 - 83.3 kg/3,250 kcal = 83.4 - 90.3 kg/3,500 kcal = 90.4 - 97.2 kg. Food was delivered to participants' home address on two occasions and labelled such that four meals were provided daily, to be consumed at times specified in the participants' daily food plan. This plan ensured that food distribution throughout the day was standardised and that food was not consumed for at least 1 h following a supplement serving. Food content was analysed using dietary analysis software (Nutritics v4.108, Nutritics Ltd., Co. Dublin, Ireland), in order to confirm content against request.

#### *4.3.3.3 Assessment of peak oxygen uptake*

Peak oxygen uptake was measured on the first visit of preliminary testing. Details of the  $\dot{V}O_{2\text{peak}}$  protocol are presented in the General Methods section, 3.4.1.

#### *4.3.3.4 Muscle damaging concurrent exercise*

The concurrent exercise session was performed in the fed state, with meal timing standardised at 2 h prior to exercise, and consisted of a high intensity simulated cycling road race and a drop-jump protocol, separated by a 15 min rest period. The cycling protocol (Table 4.2) was performed using an electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA) and has previously been used to simulate the demands of cycling road racing (Vaile et al., 2008). In brief, participants completed a 10 min self-

selected warm-up including  $3 \times 3$  s sprints at 7, 8 and 9 min, before completing a total of 66 sprints of a 5, 10 or 15 s duration, with a work (W) to rest ratio of 1:6, 1:3 or 1:1. Sprints were divided into 9 sets, with a period of active recovery completed between sprints and between sets. Intensity during active recovery was maintained at 40%–50% W achieved at  $\dot{V}O_{2peak}$ . Furthermore, 9 min of sustained effort was incorporated into the trial through the performance of a TT of 2 min (after sets 3 and 6) and 5 min (after set 9) duration. During all sprints and TT efforts, participants were encouraged to complete as much work as possible and the total duration of the trial was 109 min. Heart rate was continually recorded throughout each trial using wireless telemetry (T31 transmitter, Polar Electro Ltd., Kempele, Finland) and participants were cooled with an electric fan on a standardised setting. Strong standardised verbal encouragement was provided throughout and water was made available to participants *ad libitum*.

The allotted rest period of 15 min comprised passive rest (5 min), standardised dynamic stretching (5 min) and a drop-jump briefing (5 min). Participants then performed a total of 100 drop-jumps from a height of 0.63 m, whereby they were encouraged to jump vertically with maximal force immediately upon landing. The protocol was separated into five sets of 20 drop-jumps, with 10 s rest between each jump and 120 s between each set. Strong standardised verbal encouragement was provided throughout. This protocol has consistently demonstrated a muscle damage response (Goodall and Howatson, 2008, Miyama and Nosaka, 2004) and was selected for the endurance trained cohort, due to a lower skill/technical demand compared to more applied resistance stimuli.

**Table 4.2.** High intensity simulated cycling road race; sprint frequency/duration and work to rest ratio composition (Vaile et al., 2008).

10 min warm-up (self-selected pace)		
Set Number	Sprint Frequency x	Work : Rest Ratio
	Duration	
1	12 x 5 s	1 : 6
2	12 x 5 s	1 : 3
3	12 x 5 s	1 : 1
<b>4 min Active Recovery - 2 min TT - 4 min Active Recovery</b>		
4	6 x 10 s	1 : 6
5	6 x 10 s	1 : 3
6	6 x 10 s	1 : 1
<b>4 min Active Recovery - 2 min TT - 4 min Active Recovery</b>		
7	4 x 15 s	1 : 6
8	4 x 15 s	1 : 3
9	4 x 15 s	1 : 1
<b>5 min Active Recovery - 5 min TT - 5 min Active Recovery</b>		

#### 4.3.3.5 Maximal voluntary contraction

Isometric MVC was assessed following a standardised warm-up of 5 min at 200 W on a cycle ergometer (Wattbike, Wattbike Ltd., Nottingham, UK). Participants completed three, 3 s MVCs of the knee extensors, separated by 60 s rest, using a Cybex isokinetic dynamometer (Cybex Humac Norm, Computer Sports Medicine Inc., CA, USA). In a seated position, participants initiated a leg extension action against a fixed arm, secured proximal to the ankle joint, immediately above the malleoli (Figure 4.3). Peak torque was assessed for the participants' dominant leg, at a knee joint angle of 70° from horizontal, assessed with a goniometer (Bodycare Products., Warwickshire, UK). A knee joint angle of 70° has previously been reported to be susceptible to significant loss of isometric strength

following a single bout of eccentric exercise (McHugh and Tetro, 2003). The intra-individual reliability of this measure returned a coefficient of variation of 3.8%.

#### 4.3.3.6 Counter-movement jump

The CMJ protocol was conducted immediately following the MVC assessment. Details of the CMJ protocol are presented in the General Methods section, 3.4.2 and the set up displayed in Figure 4.3.

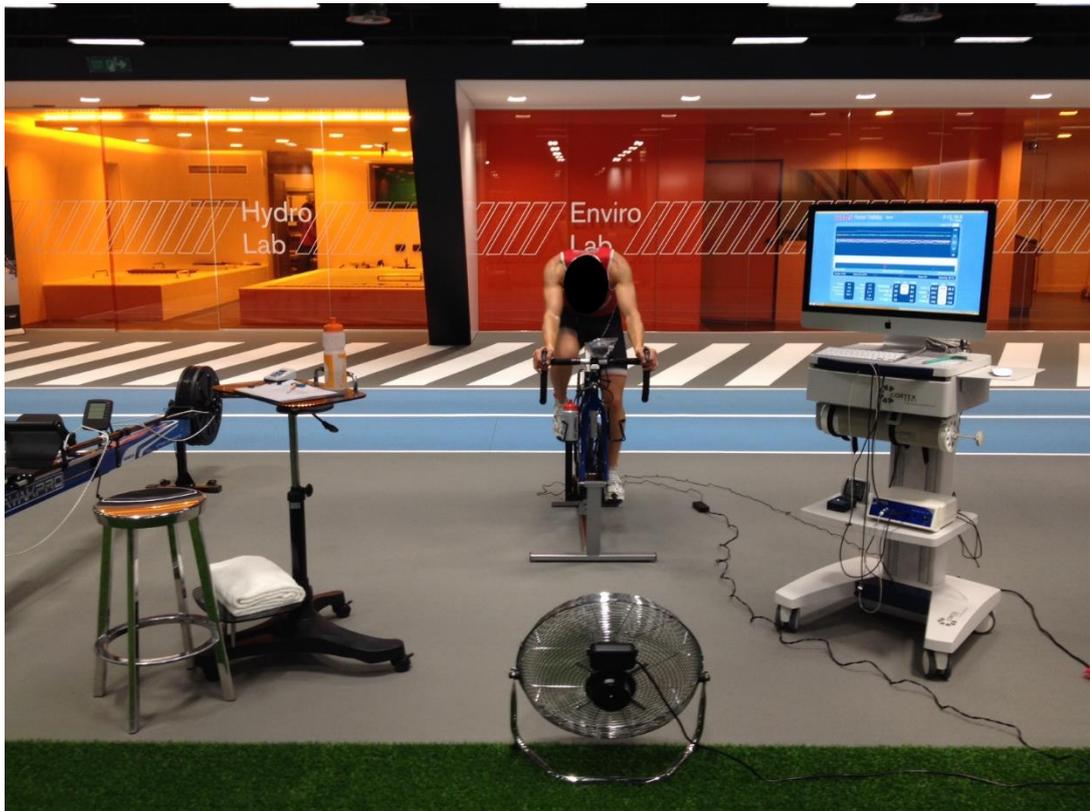


**Figure 4.3.** The experimental set up for the CMJ and MVC protocols.

#### 4.3.3.7 16.1 km time trial

Following a standardised 5 min warm-up at an intensity of 50%  $\dot{V}O_{2peak}$ , participants completed a 16.1 km TT using an electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA). The assessment required participants to complete a distance of 16.1 km in as short a time as possible, while being blinded to time elapsed (Figure 4.4). The trial started with the ergometer set in the lowest possible gear ratio, whereby after a 3 s count-down, the participant was responsible for manipulating gearing to

a desired level. Feedback of performance data was withheld, except distance elapsed, which was communicated every 1.6 km and participants were permitted to change gears as and when they felt necessary. Heart rate was continually recorded throughout each trial, using wireless telemetry (T31 transmitter, Polar Electro Ltd., Kempele, Finland) and participants were cooled with an electric fan at a standardised setting, with water available *ad libitum*. The intra-individual reliability of these measures returned a coefficient of variation 1.1% for this protocol.



**Figure 4.4.** The experimental set up for the 16.1 km time trial protocol.

#### 4.3.3.8 Muscle soreness assessment

Participants were asked to hold a fixed squat position at an  $\sim 90^\circ$  joint angle, while rating perceived muscle soreness on a 20 cm visual analogue scale, consisting of a line from 0 cm (no pain) to 20 cm (pain as bad as it could be). Using similar scales, previous

research reports significantly increased soreness following EIMD protocols (Goodall and Howatson, 2008, Mohr et al., 2016).

#### *4.3.3.9 Blood sampling and analysis*

A venous blood sample was collected from a branch of the basilica vein in the anti-cubital fossa region using venepuncture method, for assessment of indices of muscle damage (creatine kinase) and inflammation (C-reactive protein). A total of ~10 mL of blood was collected into a silica additive serum vacutainer (367896, BD Diagnostics., Dubai, UAE) and rested for 60 min to clot. The vacutainer was then centrifuged at 1300 g, 25°C for 10 min (Heraeus Multifuge 3SR Plus, Thermo Fisher Scientific Inc., MA, USA). The resultant supernatant was pipetted into aliquots and immediately stored at -80 °C, for later analysis.

Serum creatine kinase (CK) was analysed using a CK NAC-activated enzyme-linked immunosorbent assay (ELISA) kit (Randox Laboratories Ltd., County Antrim, UK). Hence, a 10 µL serum sample was mixed with 500 µL of reagent and measured at 37°C at a 340 nm wavelength, using an Rx Monza clinical chemistry analyser (Randox Laboratories Ltd., County Antrim, UK). The manufacturer reports intra-assay and inter-assay coefficients of variation for this protocol at 1.6-2.3% and 3.4-3.9%, respectively. Serum C-reactive protein (CRP) was analysed in duplicate using a Human CRP ELISA kit (R&D Systems Europe Ltd., Abingdon, UK). Hence, a 10 µL serum sample underwent a 100-fold dilution with calibrator diluent, before being processed in accordance with the manufacturer's instructions. Optical densities were determined at 540 nm and 450 nm, using a Fisher Scientific Multiskan FC Microplate Reader (Thermo Fisher Scientific Inc., MA, USA). Blank subtracted values were averaged and 450 nm readings were corrected with 540 nm values. Standard and controls were plotted as a standard curve with data linearised by producing log scales of both axes. The manufacturer reports intra-assay and

inter-assay coefficients of variation for this protocol at 3.8-8.3% and 6.0-7.0%, respectively.

#### *4.3.3.10 Statistical analysis*

Data are presented as mean  $\pm$  standard deviation (SD), with statistical significance set at  $p \leq 0.05$  a priori. Sphericity was assumed if Mauchly's test score returned  $p \geq 0.05$ , with Greenhouse-Geiser adjustments made where appropriate. All criterion measures were analysed using a group (PRO vs. CHO vs. PLA) by time-point (-24, 0, 24, 48, 72 h) repeated measures analysis of variance (ANOVA). MVC and CMJ analysis included five time-points (-24, 0, 24, 48, 72 h), while four time-points (-24, 24, 48, 72 h) were assessed for muscle soreness and blood markers, with just two time-points (-24 and 72 h) used for analysis of TT performance. Significant main effects were further investigated using LSD post-hoc, pair-wise comparisons. All data analysis was performed using statistical software (IBM SPSS 22 for Windows., New York, USA). Where appropriate, data were normalised using percentage change from baseline, to account for differences in baseline measures. Statistical power of the study was calculated using G\*Power statistical software (v3.1.9., Düsseldorf, Germany) on the basis of research investigating the effect of protein-based supplementation vs. carbohydrate control on maximal muscle function (lower-body 1-RM or peak torque during extension/flexion) at 48 h post-damage (Cockburn et al., 2008, Hoffman et al., 2010, Rankin et al., 2015), returning a hypothesised effect size of 0.3 and a subsequent sample size of 21 subjects i.e. 7 subjects per group, with  $\alpha$  set at 0.05 and sufficient statistical power of 0.8 (Cohen, 1992).

## **4.4 Results**

No differences in demographic descriptive variables between groups were observed ( $p > 0.05$ ), upon initial presentation (Table 4.3). There were no significant differences in the

prescribed energy intake, or contribution of macronutrients to daily energy intake between groups ( $p > 0.05$ ). Daily prescribed energy intake per group is listed in Table 4.4. The concurrent exercise protocol was deemed to have caused EIMD, owing to time effects ( $p < 0.001$ ), confirming decrements in MVC and CMJ performance, along with increased muscle soreness, CK and CRP. The only measure devoid of time effects was the 16.1 km TT, which was assessed at baseline and 72 h only.

**Table 4.3.** Baseline demographic descriptive data.

<b>Supp. Group</b>	<b>Age (yr)</b>	<b>Height (cm)</b>	<b>Body Mass (kg)</b>	<b><math>\dot{V}O_{2peak}</math> (ml·kg<sup>-1</sup>·min<sup>-1</sup>)</b>	<b>MVC (N·m)</b>
PRO	27 ± 3	178.8 ± 9.7	75.9 ± 8.9	62.3 ± 4.7	271.9 ± 55.9
PLA	28 ± 5	174.9 ± 5.1	72.7 ± 8.4	60.0 ± 9.0	229.8 ± 32.2
CHO	26 ± 5	178.9 ± 8.7	72.4 ± 10.0	61.2 ± 2.2	261.8 ± 50.1

**Note:** Values presented as mean ± SD. PRO = protein; CHO = carbohydrate; PLA = placebo

**Table 4.4.** Daily prescribed energy intake.

<b>Supp. Group</b>	<b>Body Mass (kg)</b>	<b>TOTAL (kcal)</b>	<b>CHO (kcal)</b>	<b>PRO (kcal)</b>	<b>FAT (kcal)</b>
PRO	75.9 ± 8.9	2813 ± 320	1821 ± 213	364 ± 43	627 ± 88
PLA	72.7 ± 8.4	2750 ± 327	1745 ± 201	349 ± 40	656 ± 111
CHO	72.4 ± 10.0	2750 ± 327	1738 ± 241	348 ± 48	665 ± 73

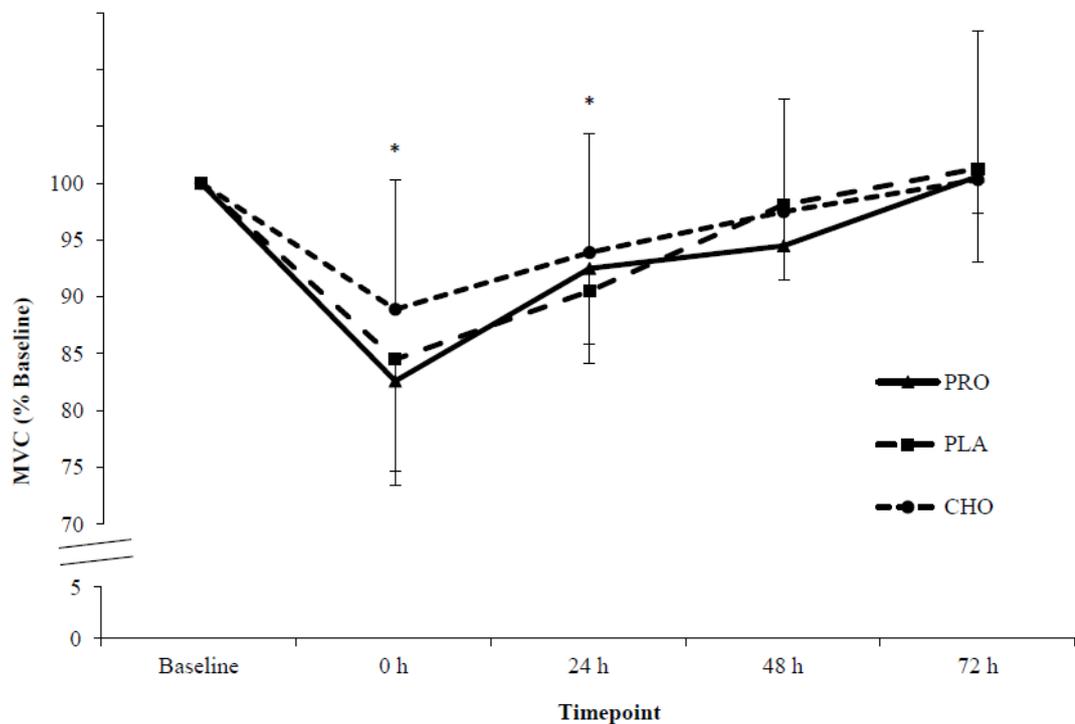
**Note:** Values presented as mean ± SD. PRO = protein; CHO = carbohydrate; PLA = placebo

#### 4.4.1 Performance measures

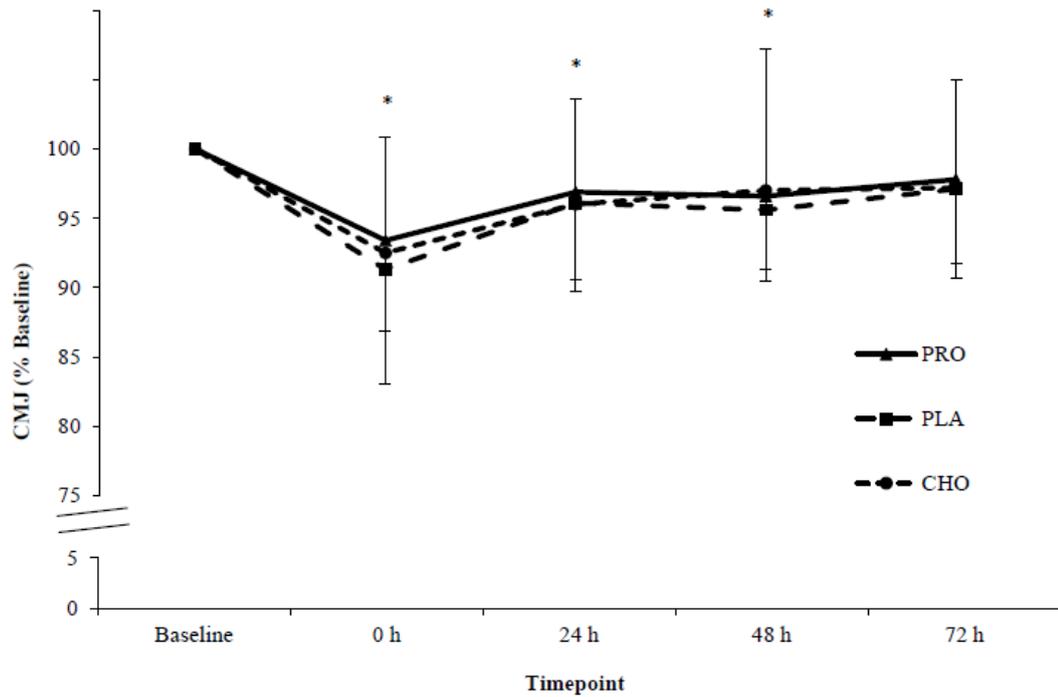
##### 4.4.1.1 Performance measures

A time effect was observed for both MVC ( $F_{[2,7,57.6]} = 30.305$ ,  $p < 0.001$ ) and CMJ ( $F_{[2,8,57.7]} = 7.342$ ,  $p < 0.001$ ), with no group or interaction effects ( $p > 0.05$ ) (Figures 4.5 and 4.6). Peak detriment for MVC was at 0 h for all groups;  $82.6 \pm 7.9$ ,  $84.5 \pm 11.1$  and

88.9 ± 11.4 % of baseline for PRO, PLA and CHO, respectively, with significant decrements from baseline at 0 and 24 h ( $p < 0.001$ ). This was consistent with CMJ performance, with all groups registering lowest values at 0 h; 93.4 ± 7.5, 91.3 ± 8.3 and 92.5 ± 5.6 % of baseline for PRO, PLA and CHO, respectively, with significant decrements from baseline at 0 ( $p < 0.001$ ), 24 ( $p = 0.032$ ) and 48 h ( $p = 0.036$ ). No time effect was observed for TT time or average  $W \cdot kg^{-1}$  sustained during the TT ( $p > 0.05$ ) and neither of these measures displayed group or interaction effects ( $p > 0.05$ ).



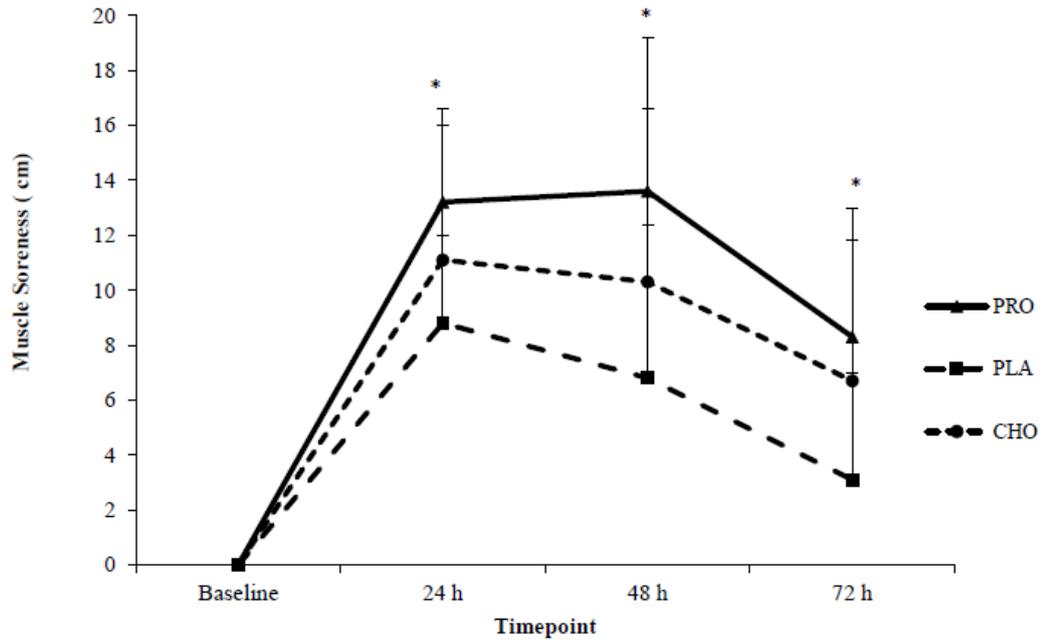
**Figure 4.5.** Isometric maximal voluntary contraction response (% change from baseline) to the concurrent exercise protocol in the PRO (n = 8), PLA (n = 8) and CHO (n = 8) groups. Absolute baseline values were 271.9 ± 55.9, 229.8 ± 32.2 and 261.8 ± 50.1 N·m for PRO, PLA and CHO, respectively. \*, significantly different from baseline ( $p < 0.05$ ). Values presented as mean ± SD. PRO = protein; CHO = carbohydrate; PLA = placebo.



**Figure 4.6.** Counter-movement jump response (% change from baseline) to the concurrent exercise protocol in the PRO (n = 8), PLA (n = 8) and CHO (n = 8) groups. Absolute baseline values were  $33.4 \pm 4.3$ ,  $33.9 \pm 2.8$  and  $32.7 \pm 3.5$  cm for PRO, PLA and CHO, respectively. \*, significantly different from baseline ( $p < 0.05$ ). Values presented as mean  $\pm$  SD. PRO = protein; CHO = carbohydrate; PLA = placebo.

#### 4.4.1.2 Muscle soreness

A time effect,  $F_{[1,7,36,6]} = 73.609$ ,  $p < 0.001$  was observed (Figure 4.7), but similar to performance measures, there was no difference between groups ( $p > 0.05$ ), nor was there an interaction between group and time ( $p > 0.05$ ). Muscle soreness was significantly elevated from baseline at all time-points ( $p < 0.001$ ).



**Figure 4.7.** Muscle soreness response to the concurrent exercise protocol in the PRO (n = 8), PLA (n = 8) and CHO (n = 8) groups. \*, significantly different from baseline ( $p < 0.05$ ). Values presented as mean  $\pm$  SD. PRO = protein; CHO = carbohydrate; PLA = placebo.

#### 4.4.1.3 Serum proteins

Both CK and CRP displayed time effects,  $F_{[1.5,27.9]} = 27.867$ ,  $p < 0.001$  and  $F_{[1.1,20.4]} = 19.765$ ,  $p < 0.001$ , respectively. However, there were no group or interaction effects ( $p > 0.05$ ) for either of these biomarkers of muscle damage and inflammation. Absolute baseline values for CK were  $258.4 \pm 103.1$ ,  $185.3 \pm 103.9$  and  $218.1 \pm 56.7$  IU·L<sup>-1</sup> for PRO, PLA and CHO, respectively. All groups displayed a similar CK profile, with peak values experienced at 24 h;  $282.4 \pm 166.2$ ,  $340.7 \pm 167.2$  and  $291.1 \pm 177.1$  % of baseline for PRO, PLA and CHO, respectively. Both CK and CRP were significantly elevated from baseline at 24 and 48 h ( $p < 0.05$ ). Absolute baseline values for CRP were  $1.13 \pm 0.66$ ,  $2.04 \pm 2.92$  and  $0.66 \pm 0.54$  mg·L<sup>-1</sup> for PRO, PLA and CHO, respectively. The time course of CRP was similar to that of CK, with peak values experienced at 24 h;  $205.6 \pm 110.4$ ,  $260.4 \pm 206.9$  and  $240.7 \pm 177.9$  % of baseline for PRO, PLA and CHO, respectively.

#### 4.4.1.4 Body mass and supplementation

The body mass reduction experienced across the trial period for the pooled participants was significant  $F_{[3,8,79.2]} = 9.399, p < 0.001$ . However, there was no difference in the body mass reduction between groups ( $p > 0.05$ );  $-0.75 \pm 0.66$ ,  $-1.08 \pm 0.70$  and  $-0.36 \pm 0.47$  kg for PRO, PLA and CHO, respectively. Upon exit questioning, none of the participants perceived to be receiving protein supplementation and only three participants noted to be unsure of which supplement condition they were receiving. Across all conditions, there was a group majority to select carbohydrate as the perceived treatment condition received (Table 4.5).

**Table 4.5.** Participant selection of perceived treatment condition at the conclusion of study participation.

Supplement Group	PLA Count	PRO Count	CHO Count	Unsure Count
PRO	3	0	4	1
PLA	0	0	7	1
CHO	2	0	5	1

**Note:** PRO = protein; CHO = carbohydrate; PLA = placebo

## 4.5 Discussion

The primary finding was that performance measures were attenuated for up to 48 h following a strenuous concurrent exercise stimulus, thereby profiling the recovery from EIMD imposed within a concurrent training paradigm. Beyond this, protein supplementation did not attenuate any of the indirect indices of EIMD.

In order to assess the efficacy of protein supplementation, it was of primary importance that the intense bout of concurrent exercise was of sufficient design to elicit a response in indirect indices of EIMD, which was suggestive of muscle damage. All five indices assessed in the 48 h following the concurrent exercise session displayed significant

time effects, indicating that the concurrent exercise bout was effective in eliciting a muscle damage response. Isometric MVC performance was reduced by a peak magnitude of 15% from baseline across the three groups, with time effects at 0 and 24 h. A review by (Clarkson and Hubal, 2002) suggests that eccentric-biased exercise protocols result in force decrements of 10-65% for between 24 h to 2 wk, depending on whether a lower or higher-force eccentric exercise protocol has been utilised. Hence, the decrement in magnitude of force and speed of return to baseline values observed in this study are at the lower end of the spectrum of reported values, compared to EIMD imposed exclusively through eccentric-biased muscle action protocols.

The muted performance decrements observed in this study could have been influenced by the metabolic challenge of the cycling protocol, with high demand on the glycolytic pathway, performed prior to the drop-jump protocol. Endurance exercise is known to significantly deplete intra-muscular glycogen levels (Noakes et al., 1988), which is associated with a concomitant reduction in the capacity to produce optimal muscular strength (Jacobs et al., 1981). Similarly, 2 h of cycling at 65% maximal aerobic power reduced eccentric muscular peak torque by 14% in well-trained cyclists, with these outcomes ascribed to a decline in the neural input to the muscle and peripheral mechanisms (Lepers et al., 2000). If the maximal force output of the muscle was compromised due to the cycling protocol, it is feasible that this would compromise the subsequent drop-jump performance. Further, the lack of time effect at 48 and 72 h for MVC performance might contribute to the lack of efficacy for protein supplementation. Previous research concerning eccentric muscle damage has reported the magnitude of EIMD to rise progressively from 0 to 48 h, with significance established at 48 h (Cockburn et al., 2008). Authors postulate that their observations might be related to protein metabolism research in animals; Lowe et al. (1995) displayed that the increase in protein degradation is not significant until 48 h following eccentric contraction-induced injury, with protein balance declining from 24-336 h, resulting in a negative state from pre-damage values. If this were to hold true in human

skeletal muscle, it is tenable to expect protein supplementation to display efficacy, if any, when protein metabolism is most stressed, possibly supporting recovery from 48 h onwards. As the deleterious effects in force output from the exercise stimulus were not significant and diminishing at 48 h post-exercise, it might not have imposed damage of a sufficient length of time in order to investigate the benefit of protein in the recovery process of this outcome measure.

CMJ performance declined by a peak magnitude of 8% from baseline across the three groups, with an effect for time at 0, 24 and 48 h. Using an identical drop-jump protocol, others have reported varying peak decrements in CMJ performance, lasting up to 96 h; ~8% (Howatson et al., 2012) and ~25% (Miyama and Nosaka, 2004). It is feasible that the discrepancies in magnitude and resolution of CMJ performance might be explained in part by participant demographics, with Howatson et al. (2012) using a population regularly competing and Miyama and Nosaka (2004) observing an un-trained population. The training history of the participants might represent a conditioning bout of exercise and lead to a reduction in the resolution and magnitude of EIMD indices in subsequent bouts, as has been discussed previously (McHugh, 2003). The repeated bout effect, facilitates a faster recovery of muscle function following a superseding bout of damaging exercise for up to 9 months (Nosaka et al., 2001), and might help to explain these findings. This is especially true given that evidence suggests a prophylactic effect even when less-severe damaging exercise is performed prior to a more severe bout of damaging exercise (Clarkson and Tremblay, 1988). It should however be noted, that the participants in this research were a well-trained cohort of endurance cyclists and as such, are unlikely to have benefited from the repeated bout effect. Exclusion criteria restricted individuals that had previously participated in a study involving activity to elicit EIMD, however participants were not excluded on the basis of strength training history. A more tenable explanation for the muted decrement in CMJ performance could be the preceding concentric work in the cycling trial. Nosaka and Clarkson (1997) reported an attenuation of muscle damage from 12 maximal

eccentric actions, when preceded by 100 repetitions of concentric muscle actions. This attenuation was evident in several indices of muscle damage; including force output, muscle soreness, and CK activity.

Perceived muscle soreness was significantly elevated from baseline at all time-points, with peak values expressed at 24 to 48 h. This response, with peak soreness of ~55% of maximum across all groups, is similar to that observed in the literature. Cockburn et al. (2008) reported a peak soreness of ~70% of maximum with time effects still apparent at the final time-point of 48 h, while others have reported peak values of ~60% of maximum soreness and time effects through to 72 h (Jackman et al., 2010, Rankin et al., 2015). An average peak CK value of  $610 \text{ IU}\cdot\text{L}^{-1}$  was observed at 24 h, across the three groups. This response in CK is consistent with other literature profiling recovery from EIMD with an eccentric muscle action bias;  $539 \text{ IU}\cdot\text{L}^{-1}$  (Cockburn et al., 2008),  $757 \text{ IU}\cdot\text{L}^{-1}$  (Green et al., 2008),  $974 \text{ IU}\cdot\text{L}^{-1}$  (Miyama and Nosaka, 2004). The range in these reported values is likely explained by variation in the participant characteristics and protocol used to induce muscle damage, with the highest CK values observed in work by Miyama and Nosaka (2004) which used a protocol of the highest volume, in a large muscle mass, in a student cohort with little or no training experience.

The elevated levels of CK in blood is indicative of disruption to the muscle membrane, with peak values normally evident no earlier than 24 h post-exercise (Warren et al., 1999). This increase in 'leakage' of intramuscular proteins into the bloodstream is just one of the events that are observed following EIMD, which appear as part of the cascade of events following the disruption of the intracellular  $\text{Ca}^{2+}$  homeostasis (Gissel and Clausen, 2001). The increased CRP values, which peaked at 24 h, suggest that the exercise stimulus was also sufficient to elicit an inflammatory response. Observations from biopsied muscle samples that have undergone eccentric muscle damage, suggest the addition of new sarcomeres and de novo synthesis in response to the stimulus (Yu et al., 2004, Yu et al., 2003). This remodelling of the muscle, with the associated reduction in contractile protein

content (Warren et al., 2002, Willoughby et al., 2003) is suggestive of an environment that would drive a requirement for protein uptake. Hence, the response in both CK and CRP subsequent to the concurrent exercise bout is suggestive of a stimulus that would place demands on protein availability, despite the lack of group effects reported in this study.

It seems logical that the smaller responses in EIMD indices in this study, particularly MVC and CMJ performance, provide a smaller window for an intervention to display efficacy. For example, if the magnitude of damage is small, then the opportunity to observe the effect of an intervention is small. Similarly, if the duration of performance decrement is acute, when investigating a process (EIMD) which possibly stresses protein metabolism maximally from 48 h onwards (Lowe et al., 1995), the opportunity to observe the effect of supplementation is reduced. Hence, the muted responses in this study, regardless of reason, might act to reduce the possibility of observing any potential beneficial effect of protein supplementation on recovery from EIMD. Further, protein supplements were provided across the observed recovery period of 72 h, with the aim of increasing bioavailability on subsequent days, whereby fractional synthesis rates remain elevated following resistance exercise (Phillips et al., 1997). This design ensured an increased quantity of supplemental protein in comparison to single bolus models in the literature, of which some have displayed efficacy for protein in supporting recovery from muscle damage (Cockburn et al., 2008, Etheridge et al., 2008). It therefore seems unlikely that the quantity of protein supplementation provided in this study would explain the lack of efficacy in supporting the recovery process.

Much of the research relating to EIMD suffers from a limitation, which is often acknowledged, in that there is a lack of control concerning habitual diet (Cockburn et al., 2008, Howatson et al., 2012, White et al., 2008). This is problematic on two counts; with underlying discrepancies in macronutrient intake affecting bioavailability of a given substrate and a likely variance in the energy balance within or between experimental groups. Activating the mTORC1 signalling pathway is a pertinent outcome of resistance

exercise, owing to its regulatory role in muscle protein remodelling (Drummond et al., 2009, Philp et al., 2011b). A recent review has highlighted the direct or indirect affect that nutrition can have on the known inputs that regulate mTORC1; amino acids, glucose, and growth factors (Drummond et al., 2009). This is supported by evidence that essential amino acids have a stimulatory effect on muscle protein synthesis (Groen et al., 2015) and the observation that carbohydrate ingestion post-resistance exercise acts to reduce protein breakdown (Borsheim et al., 2004). Hence, control of nutrient intake is of the utmost importance when conducting research involving muscle remodelling as part of the recovery process. Great effort was taken in an attempt to account for some of the limitations existing in this field of research, with a habitual diet designed in line with ACSM recommendations provided to participants' home addresses. A small, but significant reduction in body mass across all subjects was observed in this study, despite the prescription of a diet that was intended to facilitate energy balance. It is not possible to compare the response in body mass change with data from the literature, as this outcome is seldom reported. It could be argued that this data would be insightful in designs possessing discrepancies in energy content between supplemental conditions (Cockburn et al., 2008, Hoffman et al., 2010). Such designs, where participants might have experienced a significant energy deficit and one experimental group has not been allocated to an iso-caloric supplement control, would likely damage the construct validity of research aiming to investigate the efficacy of protein supplementation.

The only outcome measure devoid of a significant time effect was 16.1 km TT performance, which was only repeated at 72 h, in order to mimic the applied scenario of completing a heavy mid-week training session (0 h) prior to weekend competition (72 h). Hence, any negative effects of the concurrent damaging exercise are resolved by 72 h post-exercise and do not negatively affect 16.1 km TT performance. Given the desire to increase ecological validity with the experimental design, it was imperative that a well-trained cohort were recruited ( $\dot{V}O_{2peak}$  of 61.2 ml·kg<sup>-1</sup>·min<sup>-1</sup>). However, with regards to application,

it should be acknowledged that although the 16.1 km TT performance measure was assessed at time-points to mimic the applied scenario of completing a heavy mid-week training session prior to competition, the exercise events from baseline through to 72 h would not constitute a regular training week. This should be taken into consideration when deciding whether the observations from this research should inform an athlete's approach to recovery from an intense bout of concurrent exercise.

These data profile the response to a strenuous concurrent training stimulus and offer new information regarding the recovery from muscle damaging exercise imposed with a concurrent exercise paradigm, in a well-trained cohort. They also fail to support the efficacy of protein supplementation in attenuating the relatively modest indices of EIMD imposed by concurrent exercise, when employing great rigour around the provision of a quality habitual diet which met protein intake recommendations and the provision of appropriate supplemental controls.

## **4.6 Summary**

This study examined the response to a strenuous bout of concurrent exercise, amongst endurance-trained cyclists. Specifically, the profile of recovery within an EIMD context was observed and whether nutritional supplementation strategies would act to better support this process. The primary finding was muted performance decrements in comparison to those from the single mode EIMD literature. It was postulated that this finding might have been influenced by the metabolic challenge of the cycling protocol, performed prior to the resistance exercise stimulus. Further, the data failed to support the efficacy of protein supplementation to benefit the recovery process. This could have been as a result of the provision of a quality habitual diet and/or appropriate supplemental controls, or indeed, the relatively modest indices of EIMD imposed by the concurrent exercise. This work addresses a specific aim of the thesis, by increasing our understanding

of the responses to an intense concurrent training stimulus, amongst an endurance-trained cohort. This chapter raises the question of whether the sequence of concurrent exercise is able to modify the response to training stimuli. Therefore, the subsequent chapter will aim to address this question, with a systematic review and meta-analysis examining the role of exercise sequence within the context of the concurrent training interference effect.

**5. THE ROLE OF ACUTE  
EXERCISE SEQUENCE IN THE  
PROVISION OF THE  
INTERFERENCE EFFECT: A  
SYSTEMATIC REVIEW WITH  
META-ANALYSIS**

## 5.1 Abstract

This study examined, with a systematic review and meta-analysis, the role of exercise sequence within the context of the concurrent training interference effect. More specifically, the aim was to determine whether intra-session exercise sequence affected the outcomes of lower-body dynamic and static strength, lower-body power and muscle hypertrophy, maximal aerobic capacity, and body fat %. Given the potential for exercise sequence to influence an interference effect and the equivocal nature of the body of evidence, it was deemed important to perform a robust systematic review and meta-analysis, to provide greater clarity. Ten studies were identified from a systematic review of the literature, for the outcomes of lower-body dynamic and static strength, lower-body hypertrophy, maximal aerobic capacity, and body fat %. Each study examined the effect of intra-session exercise sequence on the specified outcomes, across a prolonged ( $\geq 5$  wk) concurrent training programme in healthy adults. Analysis of pooled data indicated that a resistance-endurance exercise sequence had a positive effect for lower-body dynamic strength, in comparison to the alternate sequence (weighted mean difference: 6.91% change; 95% CI: 1.96, 11.87% change;  $p = 0.006$ ), with no effect of exercise sequence for lower-body muscle hypertrophy (weighted mean difference: 1.15% change; 95% CI: -1.56, 3.87% change;  $p = 0.40$ ), lower-body static strength (weighted mean difference: -0.04% change; 95% CI: -3.19, 3.11% change;  $p = 0.98$ ), or the remaining outcomes of maximal aerobic capacity and body fat % ( $p > 0.05$ ). These results indicate that intra-session exercise sequence is an important training variable within the context of the interference effect, with a resistance-endurance exercise order proving beneficial for improvements in lower-body dynamic strength. There was no support for a given exercise order across a concurrent training programme for the other outcomes assessed. Given that an order effect was only observed for one outcome, it was recommended that individuals limited by time, such that they must train concurrently with minimal relief between modes of exercise, follow a resistance-endurance exercise order.

## 5.2 Introduction

Performance in many professional sports necessitates the athlete to develop muscular strength and endurance simultaneously; this dichotomous paradigm poses a challenge to optimise physiology adaptation. Hickson (1980), first reported the ‘interference effect’, attenuated strength development during a concurrent training model, in comparison to that following isolated resistance training. Given the necessity for numerous elite sporting populations to develop strength and aerobic capacity simultaneously, a significant demand has been placed upon the practice of effective concurrent training methods. This demand is also true of recreational exercisers with little time available to train, therefore completing both types of exercise in a single training session. Concurrent training is defined as the simultaneous integration of both resistance and endurance exercise within a coherent training plan (Fyfe et al., 2014). Establishing effective training methods within a concurrent exercise paradigm requires practitioners to manipulate acute training variables in order to elicit targeted adaptations for a given training cycle or intervention period. The effectiveness of the training programme therefore rests on the intricacies of manipulating exercise frequency, sequence, intensity, duration and mode.

A meta-analysis by Wilson et al. (2012) provided a quantitative approach to investigating the existence of the interference effect, using data from 21 studies. Decrements in adaptation for strength, power and hypertrophy across a training programme were observed in the concurrent groups *vs.* the resistance training groups, however these responses were only significantly blunted for power (Wilson et al., 2012). Conversely, numerous investigations have failed to evidence an interference effect on hypertrophy when comparing concurrent training with resistance training in isolation (Murach and Bagley, 2016), while some authors have reported concurrent training to augment muscle growth, but not strength, relative to resistance training alone (Lundberg et al., 2013, Lundberg et al., 2014, Mikkola et al., 2012). It is possible to adopt a somewhat myopic view of the

concurrent training paradigm, whereby the addition of an endurance stimulus is fatal to strength, hypertrophy, and or power. Instead, it is of interest to manipulate training variables, in search of an optimum adaptation for given training load and performance demands.

Investigations to identify mechanisms underpinning the potential interference effect followed the seminal work of Hickson (1980). Residual fatigue was initially theorised to provide a possible explanation for the interference effect, because of the decline in strength adaptation occurring in the latter stages of the training programme in the concurrent group, relative to the resistance training group, with a further suggestion that biochemical processes of adaptation might prove a mechanistic reason for these observations (Hickson, 1980). Subsequent work has offered additional possibilities to explain the interference effect, such as sub-optimal intra-muscular glycogen levels post-endurance exercise acting to hinder the quality of subsequent resistance exercise (Jacobs et al., 1981), or the capacity for prior endurance exercise to reduce muscular peak torque via a decline in the neural input to the muscle and peripheral contractile mechanisms (Lepers et al., 2000), or indeed, antagonistic processes at the molecular level which inhibit the potential for strength adaptation (Fyfe et al., 2014).

Regardless of the mechanism(s) underpinning the interference effect, which seem to be complex and potentially multifactorial, the role of exercise sequence has become a pertinent issue. If the interference effect does exist and athletes are required to train concurrently, it is important for the practitioner to understand the consequences of manipulating the acute training variables of exercise frequency, sequence, intensity and mode. The role of intra-session exercise sequence has been investigated in both acute and chronic scenarios, via molecular signalling response post-exercise (Coffey et al., 2009a, Coffey et al., 2009b, Jones et al., 2015) and monitoring morphological and functional outcomes following training (Davitt et al., 2014, McGawley and Andersson, 2013, Okamoto et al., 2007). A resistance-endurance exercise order throughout a training

programme has been reported to result in increased strength (Cadore et al., 2013, Okamoto et al., 2007, Pinto et al., 2015) and hypertrophy (Pinto et al., 2014), in comparison to the exercise order of endurance preceding resistance training. However, other research has found no advantage to this exercise sequence for either strength, hypertrophy, or power (Chtara et al., 2008, MacNeil et al., 2014, McGawley and Andersson, 2013). Consequently, the research is far from unequivocal and the message for athletes and practitioners is not clear. Additional work is therefore warranted to elucidate the exercise sequence effects in a concurrent exercise paradigm.

Given the potential for the order effect to influence an interference effect and the apparent equivocal nature of the body of evidence, the purpose of this work was to examine, with a systematic review and meta-analysis, the role of exercise sequence within the context of the concurrent training interference effect. More specifically, the aim was to determine whether intra-session exercise sequence affects the outcomes of lower-body dynamic and static strength, lower-body power and muscle hypertrophy, maximal aerobic capacity, and body fat %.

## **5.3 Methods**

This meta-analysis was conducted in accordance with the recommendations and criteria of the Cochrane Collaboration (<http://uk.cochrane.org/>), in line with the criteria set out in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The respective procedures were agreed upon ahead of data analysis.

### *5.3.1 Criteria for study eligibility: studies and subjects*

To be eligible for inclusion in the original article acquisition, a study had to compare the effects of exercise sequence within a concurrent training paradigm, on at least

one outcome measure of strength, power, or hypertrophy. These measures are susceptible to decrements consistent with the concurrent interference effect (Hickson, 1980, Wilson et al., 2012). Maximal aerobic capacity, defined as maximum oxygen uptake ( $\dot{V}O_{2max}$ ), and body fat % were analysed as supplementary outcomes if monitored in studies that qualified for inclusion on the grounds of strength, power, or hypertrophy outcomes. To limit the research question to the affect of within-session concurrent exercise sequence, only designs with minimal relief between modes of exercise ( $\leq 15$  min) were included, thereby excluding designs where both modes of exercise were not performed within close proximity to one another. Search criteria were not restricted on the basis of sex or training status; however, participants had to be reported as healthy and above 16 years of age, forming groups that were of similar training status at the onset of the study (e.g. both trained or untrained).

Studies containing at least two groups, allowing for the comparison of resistance followed by endurance exercise, or vice versa across a prolonged concurrent exercise training programme, were considered for inclusion. The concurrent exercise-training programme had to include at least 2 d of concurrent exercise sessions per week, across a continuous period of at least 5 wk of training. Outcome measures accepted for lower-body maximal strength capacity were separated into dynamic and static methods. Improvements relating to dynamic strength were limited to measurements of 1-RM in a variation of the squat, leg press, or leg extension exercise. Maximal isometric force recorded against an external resistance was accepted as a measure of static strength. Study inclusion for the outcome of muscle hypertrophy was limited to measurements of muscle fibre CSA by histochemical analysis or measures of whole muscle volume or thickness by magnetic resonance imaging or ultrasound, respectively. Maximal immediate power, expressed in a dynamic movement (e.g. CMJ) was required for inclusion on the basis of power. Aerobic capacity was determined by measurement of peak oxygen consumption during, or maximal workload at the end of an incremental test to volitional exhaustion. Body fat % measures were limited to dual-energy x-ray absorptiometry (DXA) scans or skinfold techniques. All

of the targeted outcome measures are reported widely in the literature, with good validity and reliability data (McCall et al., 1996, Reeves et al., 2004, Verdijk et al., 2009).

### *5.3.2 Information sources and search strategy*

In line with the Cochrane Collaboration methods, a PICO strategy was used to build search criteria for electronic database searches. PICO relates to the components of population, intervention, comparison, and outcome. To avoid database bias, a total of four databases were used; PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), Web of Science (<http://wok.mimas.ac.uk/>), MEDLINE (<http://ovidsp.tx.ovid.com>), and Science Direct ([www.sciencedirect.com/](http://www.sciencedirect.com/)). Database searches were performed in February 2016 and limited to the year 1980 onwards, from the publication of the seminal research relating to the concurrent interference effect (Hickson, 1980). The search strategy is presented in Table 5.1. Searches for unpublished data were completed on trial registries (<https://clinicaltrials.gov/> and <https://www.clinicaltrialsregister.eu/>). Following this, a primary exclusion was conducted based on an appraisal of study abstracts. In addition, supplementary searches were conducted by consulting key reviews in the field, along with a search of the reference lists in all articles found. A secondary exclusion was then conducted based on a review of full-text articles. Only studies reported in English-language sources were included. Articles were also scanned for possible duplication and contact with authors was made where duplication of results was possible.

**Table 5.1.** PubMed search strategy performed on 5<sup>th</sup> February 2016. The entry column details specific search terms entered into databases, to identify research aligned to the PICO search strategy.

Concept search strategy	Line N <sup>o</sup>	Entry
Trained/untrained	1	train*
	2	athlete*
	3	recreational exercise*
	4	“athletic performance/physiology”[Mesh]
	5	1or2or3or4
Concurrent exercise	6	concurrent exercise*
	7	concurrent training*
	8	combined training*
	9	6or7or8
RCTs	10	randomized
	11	randomly
	12	control*
	13	training study
	14	10or11or12or13
	15	5and9and14

**Note:** RCTs = randomised controlled trials. Results limited to 1980 onwards (to account for seminal research)

### 5.3.3 Study selection and data processing

A study was excluded if it compared exercise sequence without controlling for relief between different modes of exercise, or imposed a design which presented nutritional imbalances between groups. Data processing required % mean change ( $\pm$  SD) for both groups following the intervention period. These data were acquired for each outcome measure of interest, along with subject numbers in each experimental group. The primary

author read the full-text of all studies selected for entry into the meta-analysis (18 studies) and independently extracted data into a pilot form, where data were reported appropriately (3 studies). The primary author then contacted researchers from the remaining studies to request the data in the required format, or to ask for further information on study methods. A second author was responsible for independent appraisal of study selection and data extraction, with any disagreements referred to a third author for a final decision. In order to provide an indication of whether publication bias was present, funnel plot symmetry was assessed for each outcome measure, while the  $I^2$  statistic was used to quantify inconsistency across studies.

Mean difference was calculated for each study by comparison of mean % change from pre to post-intervention for each experimental group i.e. resistance followed by endurance exercise or endurance followed by resistance exercise. The SD of the mean change was also collected to enable the generation of forest plots with study-specific point estimates and respective 95% confidence intervals. The analyses of the pooled data were conducted with a fixed-effects model, where weighting was attributed based on inverse variance. Where the  $I^2$  statistic was  $\geq 50\%$ , a random-effects model was used to account for the high heterogeneity. All calculations were performed using Review Manager (RevMan, Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

#### *5.3.4 Quality assessment*

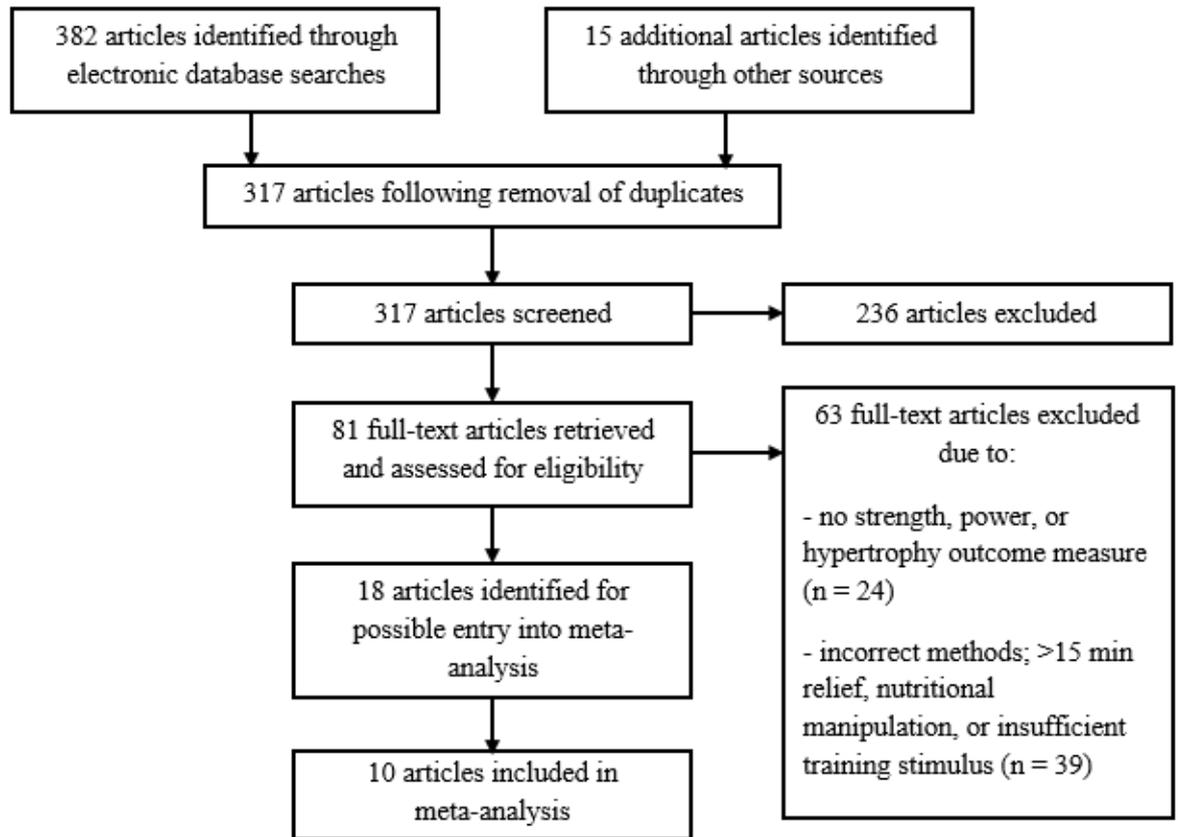
The quantitative assessment tool ‘QualSyst’ was used to assess methodological quality (Kmet et al., 2004). The tool contains 14 items which are scored depending on the degree to which specific criteria were met (yes = 2, partial = 1, no = 0), while items that are not applicable were marked “NA”. A summary score was calculated for individual studies by summing the total score obtained across relevant items and dividing it by the total possible score. A score of  $\geq 75\%$ , 55-75%, and  $\leq 55\%$  indicated strong, moderate, and weak

quality, respectively. Two reviewers independently performed quality assessments, with any disagreements referred to a third author for consensus.

## **5.4 Results**

The database searches using PubMed, Web of Science, MEDLINE, and Science Direct returned 129, 99, 90, and 64 articles, respectively, with 81 full-texts retrieved and 18 studies selected for possible entry into the meta-analysis. Despite the common theme of observing the effect of manipulating exercise sequence within a concurrent training programme, the included studies had slightly different aims; three studies focussed exclusively on applied training outcomes (Chtara et al., 2008, Collins and Snow, 1993, McGawley and Andersson, 2013), while four studies were focussed on the neuromuscular adaptations to training (Cadore et al., 2013, Eklund et al., 2015, Pinto et al., 2014, Pinto et al., 2015), with the remaining studies aiming to investigate the response in hormone concentrations (Eklund et al., 2016), vascular function (Okamoto et al., 2007), or gene expression (MacNeil et al., 2014), following alternate concurrent exercise sequences.

The call to authors resulted in confirmation of duplicated results (3 studies), ineligible research (2 studies), destroyed data (1 study), and non-responders (2 studies), leaving 10 studies suitable for inclusion in the meta-analysis. Hence, a total of 10 studies, including results from 20 groups, met all of the inclusion criteria and were included in the review (Figure 5.1). This incorporated a total population size of 227 subjects for lower-body dynamic strength, 155 subjects for lower-body static strength, 137 subjects for lower-body muscle hypertrophy, 167 subjects for body fat %, and 184 subjects for maximal aerobic capacity. The publication dates ranged from 1993 to 2016. Quality assessments of these 10 studies determined that seven were of strong quality and three were of moderate quality (Table 5.2).



**Figure 5.1.** Flow diagram of study screening process

**Table 5.2.** Quality assessment ‘QualSyst’ (Kmet et al., 2004)

Study	Question described	Appropriate study design	Appropriate subject selection	Characteristics described	Random allocation	Investigator -blinded	Subject-blinded	Outcome measures well defined and robust to bias	Sample size appropriate	Analytic methods well described	Estimate of variance reported	Controlled for confounding	Results reported in detail	Conclusion supported by results	Rating	
Cadore et al. (2013)	2	2	2	2	1	2	NA	2	2	2	2	2	2	2	2	Strong
Chtara et al. (2008)	2	2	2	1	0	0	NA	2	1	2	1	1	2	2	2	Moderate
Collins and Snow (1993)	2	2	1	2	1	0	NA	1	2	2	2	1	2	2	2	Strong
Eklund et al. (2015)	2	2	2	2	0	0	NA	2	2	2	2	2	2	2	2	Strong
Eklund et al. (2016)	2	2	2	2	0	0	NA	1	2	2	2	2	2	2	2	Strong
MacNeil et al. (2014)	2	2	2	2	1	0	NA	1	1	2	1	1	2	2	2	Moderate
McGawley and Andersson (2013)	2	1	2	2	0	0	NA	1	1	1	2	1	2	1	1	Moderate
Okamoto et al. (2007)	2	2	2	2	1	1	NA	1	2	1	1	1	2	2	2	Strong
Pinto et al. (2014)	2	2	2	2	1	2	NA	1	2	2	2	2	2	2	2	Strong
Pinto et al. (2015)	2	2	2	2	1	2	NA	1	2	2	2	2	2	2	2	Strong

**Note:** NA = not applicable, 2 = yes, 1 = partial, 0 = no, Strong = strong quality ( $\geq 75\%$ ), Moderate = moderate quality ( $55 \leq 75\%$ ), Weak = weak quality ( $\leq 55\%$ )

#### *5.4.1 Study characteristics*

Data were sourced from a total of 245 subjects with a mean age of  $31 \pm 16$  y, where 2 studies observed older ( $>55$  y) subjects and 8 studies were conducted in younger ( $<30$  y) subjects (Table 5.3). Of the 10 studies, 1 study was conducted in professional athletes, 3 studies observed recreationally active cohorts, while 6 studies were conducted in untrained subjects. The 20 groups in the analysis were comprised of male (8 groups), female (6 groups), and mixed (6 groups) cohorts.

#### *5.4.2 Publication bias and inconsistency*

Effect estimates in the studies with smaller standard error were closer to the true intervention odds ratio, while symmetry was observed upon visual inspection of each outcome measure funnel plot, indicating no clear evidence for publication bias. It must be noted however, that this provides no guarantee that the analysis is free from publication bias (Lau et al., 2006). Of the five outcome measures, calculated  $I^2$  statistics were as follows: 66% for lower-body dynamic strength, 17% for lower-body static strength, 72% for lower-body muscle hypertrophy, 11% for body fat %, and 0% for maximal aerobic capacity. In line with the Cochrane Collaboration thresholds, values up to 60% represent the possibility of moderate heterogeneity, while values up to 90% might represent substantial heterogeneity.

**Table 5.3.** Characteristics of the individual studies included in the meta-analysis

Study	Mean age (y)	Training status	Concurrent training details					
			Study length	Training frequency	Relief duration	RES volume range sets x reps (intensity)	END modality	END duration (intensity)
Cadore et al. (2013)	65	Untrained	12 wk	3 d/wk	<10 min	2-3 x 6-20 (48-93% 1-RM)	Cycling	20-30 min (80-95% HR <sub>VT</sub> )
Chtara et al. (2008)	21	Recreational	12 wk	2 d/wk	<15 min	4-5 x 5-32 (circuit training)	Running	Variable ( $\dot{V}O_{2max}$ intervals)
Collins and Snow (1993)	22	Untrained	7 wk	3 d/wk	None	2 x 3-12 (50-90% 1-RM)	Running	25 min (60-90% HRR)
Eklund et al. (2015)	29	Recreational	24 wk	2 d/wk - 5 d/2 wk	<10 min	2-5 x 3-20 (40-95% 1-RM)	Cycling	25-50 min (~AT/at or >AnT)
Eklund et al. (2016)	29	Recreational	24 wk	2 d/wk - 5 d/2 wk	<10 min	2-5 x 3-20 (40-95% 1-RM)	Cycling	30-50 min (~AT/at or >AnT)
MacNeil et al. (2014)	20	Untrained	6 wk	3 d/wk	None	3 x 10 (65-80% 1-RM)	Cycling	22.5 min (65-75% $\dot{V}O_{2max}$ )
McGawley and Andersson (2013)	23	Trained	5 wk	3 d/wk	<5 min	2-3 x 4-20 (75-90% 1-RM)	Running (football-specific)	30 min (90-95% HR <sub>max</sub> intervals)
Okamoto et al. (2007)	18	Untrained	8 wk	2 d/wk	None	5 x 8-10 (80% 1-RM)	Running	20 min (60% THR)
Pinto et al. (2014)	25	Untrained	12 wk	2 d/wk	None	3-6 x 10-20 s (maximal effort)	Water-based	18-36 min (HR <sub>VT2</sub> )
Pinto et al. (2015)	57	Untrained	12 wk	2 d/wk	None	3-6 x 10-20 s (maximal effort)	Water-based	18-36 min (HR <sub>VT2</sub> )

**Note:** RES = resistance training, END = endurance training, reps = repetitions, 1-RM = 1-repetition maximum, HR<sub>VT</sub> = heart rate at ventilatory threshold,  $\dot{V}O_{2max}$  = maximum oxygen uptake, HRR = heart rate reserve, AT = aerobic threshold, AnT = anaerobic threshold, HR<sub>max</sub> = maximum heart rate, THR = targeted heart rate, HR<sub>VT2</sub> = heart rate at second ventilatory threshold

### *5.4.3 Intervention effects and pooled analyses*

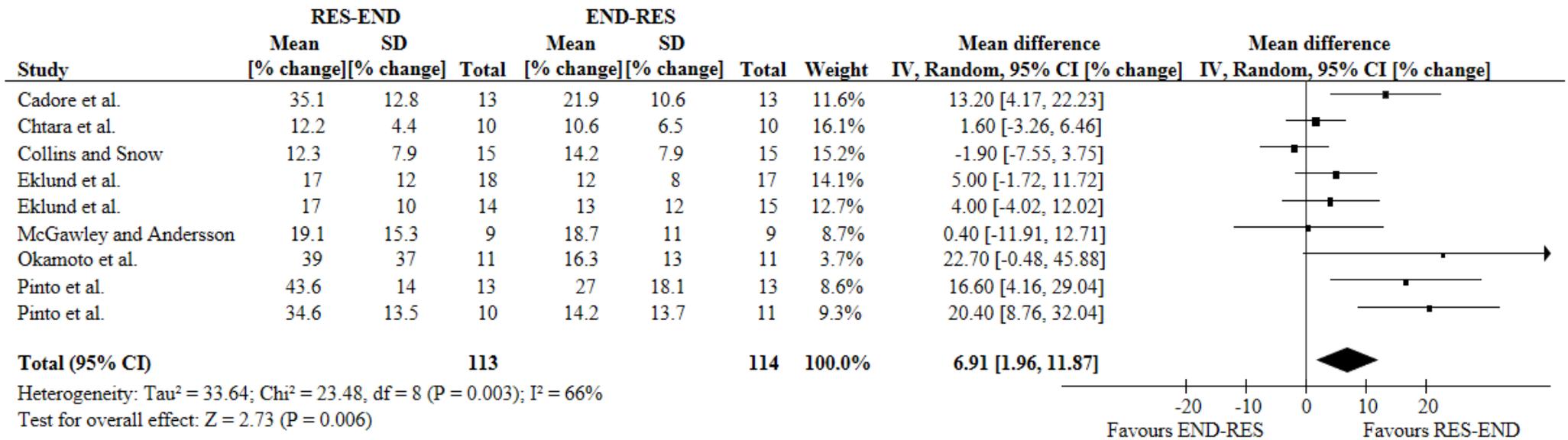
An overview of the effect from individual studies along with a 95% confidence interval is presented in Table 5.4. The % mean changes following intervention for each of the five outcome measures were individually assessed. Many of the selected publications included further outcome measures, but only those that are relevant to the review have been summarised. The range of mean difference was -1.9 to 22.7% for lower-body dynamic strength, -4.0 to 4.4% for lower-body hypertrophy, -10.0 to 5.5% for lower-body static strength, -5.4 to 1.7% for aerobic capacity, and -4.4 to 4.1% for body fat % (where a negative value favours ENDURANCE-RESISTANCE and a positive value favours RESISTANCE-ENDURANCE exercise sequence).

**Table 5.4.** Individual study results included in the meta-analysis

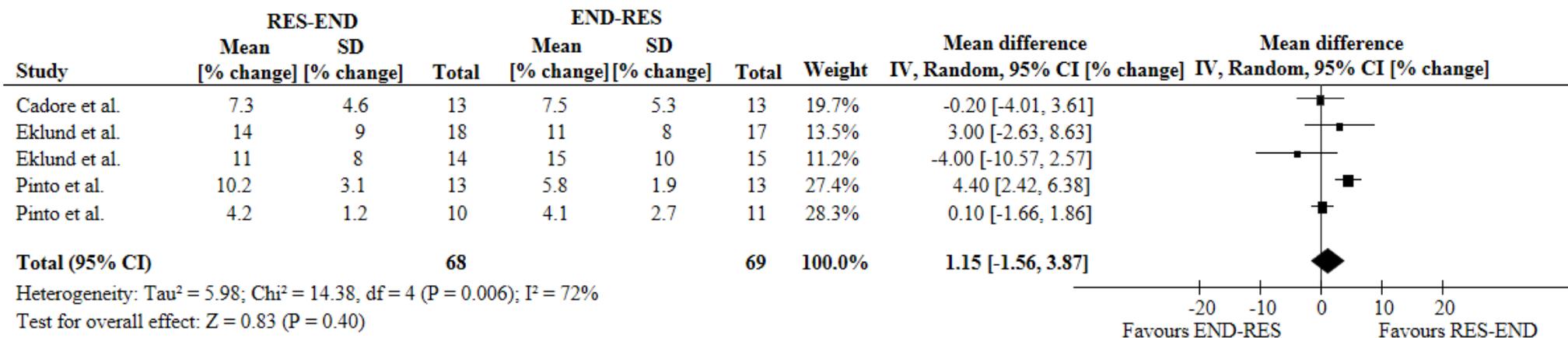
Study	Outcome measures				
	LBDS	LBSS	LBMH	BF%	MAC
Cadore et al. (2013)	RE (4.17, 22.23)	RE (-4.19, 8.79)	ER (-4.01, 3.61)	RE (-3.03, 5.23)	ER (-8.77, 6.37)
Chtara et al. (2008)	RE (-3.26, 6.46)			RE (-8.44, 8.84)	
Collins and Snow (1993)	ER (-7.55, 3.75)				RE (-4.88, 5.28)
Eklund et al. (2015)	RE (-1.72, 11.72)	RE (-9.93, 13.93)	RE (-2.63, 8.63)		
Eklund et al. (2016)	RE (-4.02, 12.02)	ER (-21.37, 1.37)	ER (-10.57, 2.57)	ER (-8.53, 8.23)	ER (-7.85, 5.85)
MacNeil et al. (2014)		RE (-5.60, 16.60)		ER (-8.54, 1.54)	ER(-12.91, 10.11)
McGawley and Andersson (2013)	RE(-11.91, 12.71)			RE (-7.05, 8.05)	
Okamoto et al. (2007)	RE (-0.48, 45.88)			RE (-0.31, 4.11)	
Pinto et al. (2014)	RE (4.16, 29.04)	ER (-11.38, 2.98)	RE (2.42, 6.38)		RE (-2.95, 6.35)
Pinto et al. (2015)	RE (8.76, 32.04)	RE (-4.16, 6.56)	RE (-1.66, 1.86)		ER (-14.70, 3.90)

**Note:** LBDS = lower-body dynamic strength, LBSS = lower-body static strength, LBMH = lower-body muscle hypertrophy, BF% = body fat %, MAC = maximal aerobic capacity. RE = outcome in the direction of performing resistance exercise first, ER = outcome in the direction of performing endurance exercise first. (.) = 95% CI

Compared with endurance followed by resistance exercise, performing resistance exercise first enhanced the improvement in lower-body dynamic strength within a prolonged concurrent-type training programme (weighted mean difference: 6.91% change; 95% CI: 1.96, 11.87% change;  $p = 0.006$ ; Figure 5.2). However, exercise sequence had no effect on lower-body muscle hypertrophy, compared to performing endurance exercise first within concurrent training sessions (weighted mean difference: 1.15% change; 95% CI: -1.56, 3.87% change;  $p = 0.40$ ; Figure 5.3).

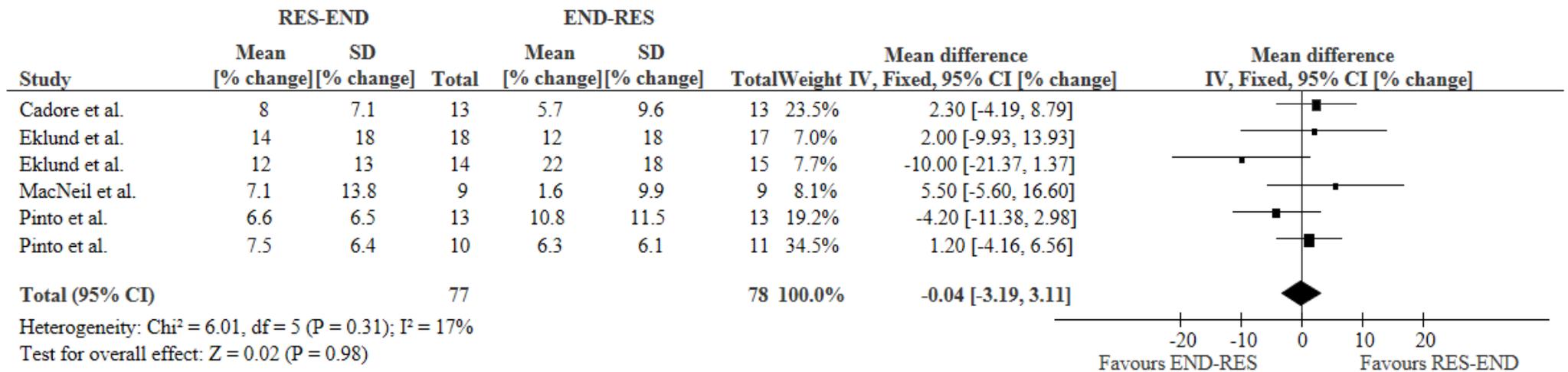


**Figure 5.2.** Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on lower-body dynamic strength (weighted mean difference: 6.91%; 95% CI: 1.96, 11.87%;  $p=0.006$ ). For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded square reflects the relative weight of the study in the meta-analysis. The shaded diamond represents the pooled mean difference. RES-END = resistance training before endurance training, END-RES = endurance training before resistance training, SD = standard deviation, IV = inverse variance, CI = confidence interval

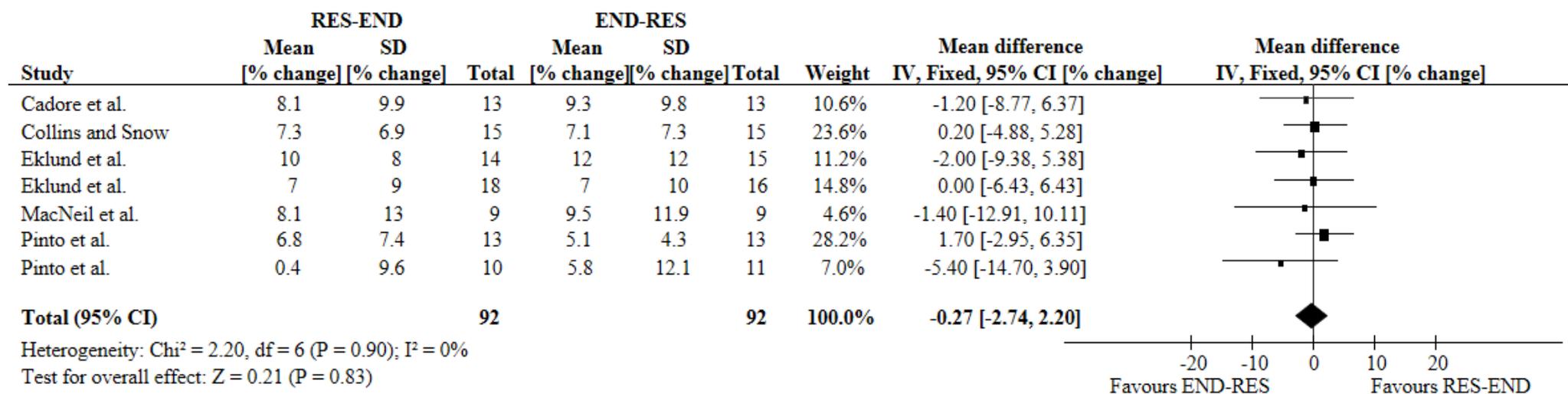


**Figure 5.3.** Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on lower-body muscle hypertrophy (weighted mean difference: 1.15%; 95% CI: -1.56, 3.87%;  $p=0.40$ ). For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded square reflects the relative weight of the study in the meta-analysis. The shaded diamond represents the pooled mean difference. RES-END = resistance training before endurance training, END-RES = endurance training before resistance training, SD = standard deviation, IV = inverse variance, CI = confidence interval

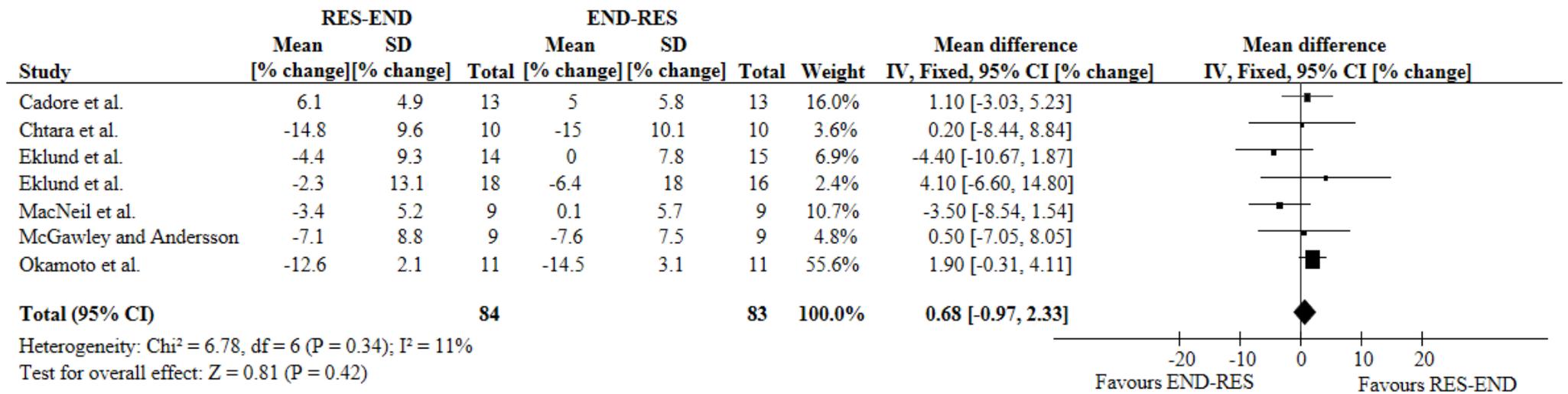
Exercise sequence had no effect on lower-body static strength (weighted mean difference: -0.04% change; 95% CI: -3.19, 3.11% change;  $p = 0.98$ ; Figure 5.4). This was also true of maximal aerobic capacity, with improvements following concurrent training not differing between contrasting orders of exercise modes (weighted mean difference: -0.27% change; 95% CI: -2.74, 2.20% change;  $p = 0.83$ ; Figure 5.5). Finally, performing endurance exercise prior to resistance exercise had no significant effect on body fat %, compared to performing resistance exercise first throughout a concurrent training programme (weighted mean difference: 0.68% change; 95% CI: -0.97, 2.33% change;  $p = 0.42$ ; Figure 5.6). There were not enough data to compare the effects of exercise sequence on lower-body power (2 studies).



**Figure 5.4.** Forest plot of the results of a fixed-effects meta-analysis shown as pooled mean differences with 95% CIs on lower-body static strength (weighted mean difference: -0.04%; 95% CI: -3.19, 3.11%;  $p=0.98$ ). For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded square reflects the relative weight of the study in the meta-analysis. The shaded diamond represents the pooled mean difference. RES-END = resistance training before endurance training, END-RES = endurance training before resistance training, SD = standard deviation, IV = inverse variance, CI = confidence interval



**Figure 5.5.** Forest plot of the results of a fixed-effects meta-analysis shown as pooled mean differences with 95% CIs on maximal aerobic capacity (weighted mean difference: -0.27%; 95% CI: -2.74, 2.20%;  $p=0.83$ ). For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded square reflects the relative weight of the study in the meta-analysis. The shaded diamond represents the pooled mean difference. RES-END = resistance training before endurance training, END-RES = endurance training before resistance training, SD = standard deviation, IV = inverse variance, CI = confidence interval. <sup>1</sup> data collected during study, but obtained through communication with author



**Figure 5.6.** Forest plot of the results of a fixed-effects meta-analysis shown as pooled mean differences with 95% CIs on body fat % (weighted mean difference: 0.68%; 95% CI: -0.97, 2.33%;  $p=0.42$ ). For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded square reflects the relative weight of the study in the meta-analysis. The shaded diamond represents the pooled mean difference. RES-END = resistance training before endurance training, END-RES = endurance training before resistance training, SD = standard deviation, IV = inverse variance, CI = confidence interval. <sup>1</sup> data collected during study, but obtained through communication with author

## 5.5 Discussion

This is the first meta-analytic review to assess the role of exercise sequence within the context of the concurrent training interference effect. Pooled estimates revealed that intra-session exercise sequence during a prolonged ( $\geq 5$  wk) concurrent training programme significantly affected the improvements in lower-body dynamic strength, with a resistance followed by endurance exercise order superior to the alternative sequence. Meanwhile, the training outcomes of lower-body static strength and muscle hypertrophy were not significantly affected by intra-session sequencing of exercise mode. Finally, maximal aerobic capacity and body fat %, outcomes which are not associated with concurrent training interference (Wilson et al., 2012), were unaffected by intra-session exercise sequence.

Evidence exists to support the concurrent interference effect, which consists of decrements in strength-based outcomes when practising this type of training relative to resistance training in isolation (Wilson et al., 2012). As such, it is of interest to observe whether the manipulation of exercise sequence can play a role in mitigating, or indeed exacerbating, this phenomenon. This was true of lower-body dynamic strength, with a resistance-endurance exercise sequence proving superior to the alternative order. Previous research suggests that a resistance followed by endurance exercise sequence is beneficial when prioritising strength-based outcomes (Cadore et al., 2013, Okamoto et al., 2007, Pinto et al., 2015), supporting the finding for lower-body dynamic strength. Interestingly, Hickson (1980) failed to report the exercise sequence of the concurrent training group. This lack of reporting prevents confirmation as to whether the primary finding of this research would act to mitigate or exacerbate the interference effect associated with concurrent training in this original research.

When contextualising the findings of this research, it is important to understand the factors which govern adaptation to contrasting types of maximal efforts. The concept of

training specificity is well established, whereby resistance training consisting of dynamic contractions results in greater improvements during isotonic vs. isometric contractions (Thorstensson et al., 1976), and hence a degree of contraction-type specificity. Further, adaptation is specific to the contraction-velocity of the training stimulus; Kanehisa and Miyashita (1983) reported maximal torques at isokinetic speeds which coincided with the contraction velocity region of the training stimulus. This work observed that strength adaptation, following a concurrent training programme, was only susceptible to modification from exercise sequence during dynamic and not static contractions. The greater increase in dynamic vs. static strength, irrespective of exercise sequence, is likely explained by the dynamic training methods of the included studies. However, the concept of training specificity fails to explain why dynamic strength was the only outcome to be modified by intra-session exercise sequence.

There is support from research investigating the order effect that the resistance stimulus should precede endurance exercise, given that residual fatigue from the alternate exercise sequence has been suggested to negatively affect the training-induced strength gains (Bell et al., 1988, Cadore et al., 2013, Pinto et al., 2015). The primary finding of this meta-analysis therefore supports this premise, given that lower-body dynamic strength adaptation was improved following a resistance-endurance exercise sequence. What is less clear is why this outcome was modified by exercise order. It is possible that the observed order effect is explained by residual fatigue, with the stress of the preceding endurance stimulus acting to hinder the quality of the resistance session. Indeed, Lepers et al. (2000) reported that 2 h of cycling at 65% maximal aerobic power reduced muscular peak torque by 14% in well-trained cyclists, with these outcomes ascribed to a decline in the neural input to the muscle and peripheral mechanisms. Cadore et al. (2013) postulated that greater adaptation in lower-body dynamic 1-RM with a resistance-endurance exercise sequence might be attributed to improved neuromuscular economy, with improvements in strength and reduced EMG activity for a given load. A suggested role for adjustments in the nervous

system is also supported by Eklund et al. (2015), with increased maximal force in combination with an increase in muscle activation in the resistance-endurance training group only. However, if residual fatigue or neuromuscular mechanisms were responsible for the observation that exercise sequence modifies the adaptation in lower-body dynamic strength, it remains to be answered why these factors would not facilitate enhanced lower-body static strength also. The finding that hypertrophy and dynamic strength outcomes were not similarly influenced by exercise sequence has been reported previously in the literature, albeit in an older population (Cadore et al., 2013).

The outcome of power is reported to be most susceptible to interference from concurrent training methods (Wilson et al., 2012), suggesting that velocity of contraction during maximal efforts might be an important factor. Concurrent training has been reported to attenuate strength adaptation in the high-velocity, low-force region of the force-velocity relationship, relative to resistance training in isolation. Resistance training in isolation improved maximal torque at angular velocities ranging from 0-4.19 rad·s<sup>-1</sup>, while improvements from concurrent training were limited to the range of 0-1.68 rad·s<sup>-1</sup>, despite both groups completing resistance training at an angular velocity of 4.19 rad·s<sup>-1</sup> (Dudley and Djamil, 1985). This is particularly important in the applied scenario, given that the majority of athletic performances require a limb speed of  $\geq 3.14$  rad·s<sup>-1</sup> (Kanehisa and Miyashita, 1983). The susceptibility of higher velocity actions to the interference effect has further support (Häkkinen et al., 2003, Lundberg et al., 2014). Häkkinen et al. (2003) reported concurrent training to result in attenuated rapid force production, relative to resistance training in isolation, possibly explained by a reduction in rapid voluntary neural activation. If high velocity contractions against resistance are most affected by the interference effect, it could perhaps be that the order effect would be most apparent during outcomes assessing maximal power, rather than isometric activity. For example, if power is most affected by the addition of endurance stimuli (Wilson et al., 2012), it would seem logical that prioritising the resistance stimulus (with a resistance-endurance exercise

sequence) would be of greater importance than for an outcome less affected by the opposing endurance stimuli. Unfortunately, there was insufficient data to include power outcomes in this meta-analysis, but generating sufficient data to analyse the order effect on higher-velocity maximal effort outcomes would be a pertinent research question to investigate in the future.

This study provides an overview of the data available on the effect of manipulating exercise sequence on the interference effect. It should be noted that while a meta-analysis does play a role in causal inference, it is not its primary purpose; rather, it provides an assessment of the consistency of results reported at an individual study level, in addition to offering greater precision of the summary effect outcomes (Weed, 2010). Some of the outcome measures reported had moderate to substantial heterogeneity, indicating a level of inconsistency in the results of individual studies. This could be a representation of the different methods utilised between individual studies, or indeed, the breadth of the age and training status in the study treatment groups. Despite symmetry in the funnel plot assessment, a publication bias risk is possible (as with all research) because of the inclusion of published articles showing positive findings and the non-publication of research which displays no effect. Further, the search of English-language sources only might have resulted in missed data. Beyond this, the role of exercise intensity within the context of the interference effect is a topical area of research (Fyfe et al., 2016a, Fyfe et al., 2016b). It is possible that the relatively untrained cohorts included in the meta-analysis are limited by their ability to perform at higher exercise intensities, and the subsequent affect that this could have on the interference effect or benefit of a given intra-session exercise order is unknown and would be justified avenues for future research. Despite the limitations, this meta-analysis provides an assessment of the potential for intra-session exercise sequence to manipulate strength-based outcomes associated with the concurrent training interference effect.

In conclusion, the findings support the practice of a resistance followed by endurance exercise order for the training outcome of lower-body dynamic strength during a prolonged ( $\geq 5$  wk) concurrent training programme. In the majority of athletic scenarios, limb movement is required and maximal dynamic strength is therefore likely of greater importance than static strength. As such, the observation for dynamic strength is likely to be meaningful to the athlete and practitioner. There was no support for a given exercise order for the training outcomes of lower-body static strength and muscle hypertrophy. This was true also of maximal aerobic capacity and body fat %. Given that an order effect was only observed for one outcome, it is recommended that individuals limited by time, such that they must train concurrently with minimal relief between modes of exercise, follow a resistance-endurance exercise order. Manipulating acute training variables might help to optimise adaptation. Given the cohorts included in this meta-analysis (and the body of evidence), the conclusions are particularly relevant to recreational exercisers or untrained individuals. Finally, while maximal aerobic capacity and body fat % are not associated with concurrent training interference (Wilson et al., 2012), it was important to observe whether they were affected by the order effect. These outcomes are often assessed following endurance interventions and their inclusion in the meta-analysis is a reminder that the concurrent training paradigm is a challenge because of the need for athletes to adapt divergent physiology in parallel.

## **5.6 Summary**

This study examined, with a systematic review and meta-analysis, the role of exercise sequence within the context of the concurrent training interference effect. More specifically, it investigated whether intra-session exercise sequence affected the outcomes of lower-body dynamic and static strength, lower-body power and muscle hypertrophy, maximal aerobic capacity, and body fat %. Given the potential for exercise sequence to

influence an interference effect and the equivocal nature of the body of evidence, it was deemed important to perform a robust systematic review and meta-analysis, to provide greater clarity. It was confirmed that intra-session exercise sequence is an important training variable within the context of the interference effect, with a resistance-endurance exercise order proving beneficial for improvements in lower-body dynamic strength. There was no support for a given exercise order across a concurrent training programme for the other outcomes assessed. Despite this, the finding for lower-body dynamic strength is pertinent, owing to its more direct application to the performance environment in comparison to some of the other outcomes assessed. This work addresses a specific aim of the thesis, by providing clarity as to whether intra-session concurrent exercise sequence modifies strength-based outcomes associated with the interference effect. This chapter emphasises that manipulating acute training variables might help to optimise adaptation to concurrent training. The subsequent chapter will aim to address this question, by examining the potential for endurance exercise intensity to modify the acute response to concurrent exercise.

**6. THE ACUTE INTERFERENCE  
EFFECT AND THE ROLE OF  
EXERCISE INTENSITY**

## 6.1 Abstract

This study investigated whether combining strength and endurance activity would result in the inhibition of anabolic signalling proteins, relative to strength stimuli performed in isolation, amongst trained endurance cyclists which were strength training naïve. Further, the study was designed to examine whether the intensity of the endurance stimulus might affect the activation status of signalling proteins associated with the mTOR and AMPK networks. Eight male, trained endurance cyclists were randomised to complete either 1) resistance exercise (RES) only; 2) resistance exercise followed by moderate intensity cycling (RES + MIC); 3) resistance exercise followed by work- and duration-matched high intensity interval cycling (RES + HIIC), in a counter-balanced order. The experimental trials required participants to complete RES only (6 x 8 squat repetitions at 80% predicted 1-RM), or an identical stimulus, followed by either MIC (40 min cycling at 65%  $\dot{V}O_{2peak}$ ) or HIIC (40 min cycling with 3 min alternating intervals of 85 and 45%  $\dot{V}O_{2peak}$ ). Muscle biopsies were collected at rest and 3 h post-RES, to assess phosphorylation of protein kinases associated with the mTOR and AMPK signalling pathways. There was a main effect of condition for the phosphorylation of mTOR<sup>S2448</sup> ( $p = 0.043$ ), with a greater response in the RES + MIC relative to RES condition ( $p = 0.033$ ). There was also a main effect of condition for the phosphorylation of AMPK $\alpha$ 2<sup>T182</sup> ( $p = 0.041$ ), with a greater response in RES + MIC, relative to both RES + HIIC ( $p = 0.026$ ) and RES ( $p = 0.046$ ). There were no other condition effects for the activation status of the remaining protein kinases assessed ( $p > 0.05$ ). Despite differential AMPK and mTOR signalling between conditions, these results indicate a lack of support for an acute molecular interference effect in a trained endurance cohort. Further, in an acute context, the data failed to support the intensity-dependent regulation of AMPK, nor differential activation of the anabolic machinery with the manipulation of endurance exercise intensity.

## 6.2 Introduction

The ‘interference effect’ describes attenuated strength development during a concurrent training paradigm, in comparison to that following isolated resistance training (Hickson, 1980). This seminal work was the first to propose an antagonistic relationship between strength and endurance adaptations. This theory has received support in subsequent years, with power adaptation particularly susceptible to concurrent training practices (Wilson et al., 2012). This conflict between opposing sides of the training adaptation continuum is troublesome for elite and recreational athletes alike.

The observed interference effect has been proposed to occur due to a molecular interference, where the process of myofibrillar protein synthesis is impeded by the cellular pathway that regulates energy production for endurance activity (Nader, 2006). Activating mTORC1 is a pertinent outcome of resistance exercise, owing to its function as a principal mediator of skeletal muscle remodelling (Philp et al., 2011a). Similarly, the phosphorylation of the protein AMPK is of key importance to the endurance adaptation training process, due to its function of monitoring the energy status of the muscle and initiating aerobic adaptive responses (Hardie, 2004, Winder et al., 2006). The logic dictates that these two pathways prove antagonistic to one another, given that a role of AMPK is to reduce energy-consuming anabolic processes within the cell (Jorgensen et al., 2006, Kahn et al., 2005). Despite this, there is evidence to the contrary (Apro et al., 2015).

Previous efforts to explore acute mechanisms have observed no inhibition of growth-related signalling (Apro et al., 2015, Apro et al., 2013, Jones et al., 2015) or an augmented strength response (Lundberg et al., 2012, Lundberg et al., 2016, Pugh et al., 2015) following concurrent stimuli, with few data providing some support for a molecular interference effect (Coffey et al., 2009a, Coffey et al., 2009b). The majority of research examining an acute interference effect has focused on studying concurrent stimuli *vs.* resistance stimuli in isolation (Apro et al., 2013, Fernandez-Gonzalo et al., 2013, Lundberg

et al., 2012), while others have manipulated the concurrent stimuli through an acute training variable, such as exercise sequence (Coffey et al., 2009b, Jones et al., 2015). There is limited research addressing the role of endurance exercise intensity and how this might be implicated in the purported acute interference effect (Fyfe et al., 2016b).

Exercise intensity is a key training variable. Given that endurance activity is purported to be antagonistic to an early growth response, it would seem logical that a greater endurance exercise intensity might exacerbate the issue. Experimentally, Rose et al. (2009) observed greater phosphorylation of AMPK indices following higher intensity cycling exercise (85%  $\dot{V}O_{2peak}$ ) vs. lower intensity exercise (35%  $\dot{V}O_{2peak}$ ), supporting the intensity-dependent regulation of AMPK. HIIT can offer adaptations consistent, if not superior to that of traditional endurance training, with regards to aerobic capacity (Gormley et al., 2008, Hwang et al., 2011, Wisloff et al., 2007) and is therefore an appealing training modality for endurance athletes. It is a method of training which can be effective in eliciting endurance adaptations in well-trained cohorts (Skovereng et al., 2018).

If endurance stimuli can impede strength adaptation signalling processes, it is suggested that a trained endurance phenotype could be of great relevance. There is strong rationale for trained endurance athletes to undertake resistance training (Rønnestad et al., 2010, Rønnestad et al., 2017). Training status is suggested to modify the early molecular signalling responses to opposing exercise stimuli, with an attenuated response amongst trained phenotypes and a generic molecular footprint in untrained cohorts (Coffey and Hawley, 2016, Coffey et al., 2006). This could suggest untrained individuals to be a poor vehicle to explore the molecular bases of an interference effect. Further, the data from Coffey et al. (2006) might suggest trained athletes to be more susceptible to an interference effect. Despite this, there are no data concerning the role of endurance exercise intensity in providing a molecular interference amongst trained endurance athletes with no strength training history. Observation of the molecular response in individuals with this concurrent training status i.e. endurance-trained but strength-naïve, will provide novel data, which

might prove valuable in better understanding the potential to induce a molecular interference.

The purpose of this study were twofold. Firstly, to examine whether combining strength and endurance activity (independent of intensity) result in the inhibition of anabolic signalling proteins, relative to strength stimuli performed in isolation. Secondly, to observe whether the intensity of the endurance stimuli might affect the activation status of signalling proteins associated with the mTOR and AMPK networks. These questions were to be answered within the context of trained endurance cyclists, that were strength training naive. This chapter will therefore address whether acute interference is evident in an endurance trained cycling cohort, and whether endurance exercise intensity plays a role in mitigating the potential effect.

## **6.3 Methods**

### *6.3.1 Design*

The study utilised a within-subject, repeated measures design. Following three preliminary trials for familiarisation to procedures and collection of subject characteristics, participants attended the laboratory on three further occasions. Participants were randomised to complete either 1) resistance exercise (RES) only; 2) resistance exercise followed by moderate intensity cycling (RES + MIC); 3) resistance exercise followed by work- and duration-matched high intensity interval cycling (RES + HIIC), in a counter-balanced order. Visits were separated by ~ 1 wk (range: 6-14 days) and participants were deemed fit for testing if they could confirm that they were free from medications/vitamin supplementation and had refrained from external exercise, caffeine and alcohol for 24 h. Participants were asked to maintain habitual diet and exercise practices throughout the duration of the study.

Preliminary data were collected for height, body mass, and  $\dot{V}O_{2\text{peak}}$ , while the remainder of the preliminary visits were used to coach for and assess 5-RM of the back squat exercise.  $\dot{V}O_{2\text{peak}}$  and 5-RM data were used to prescribe relative exercise intensities for the three experimental trials. The experimental trials required participants to complete RES only (6 x 8 squat repetitions at 80% predicted 1-RM), or an identical stimulus, followed by either MIC (40 min cycling at 65%  $\dot{V}O_{2\text{peak}}$ ) or HIIC (40 min cycling with 3 min intervals of 85 and 45%  $\dot{V}O_{2\text{peak}}$ ). The endurance exercise stimuli are presented in the General Methods section, 3.5.3. Muscle biopsies were collected at rest and 3 h post-RES, to assess phosphorylation of protein kinases associated with the mTORC1 and AMPK signalling pathways (General Methods section, 3.5.4).

### *6.3.2 Participants*

Eight male, trained endurance cyclists (age  $32 \pm 5$  years; height  $179 \pm 4$  cm; mass  $70.6 \pm 7.0$  kg;  $\dot{V}O_{2\text{peak}}$   $55.4 \pm 7.1$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) volunteered to take part in the study. Participants had no resistance training history for  $\geq 6$  months prior to enrolment. Additional information relating to the ethical approval of the study and its participants are presented in the General Methods sections, 3.2 and 3.3, respectively.

### *6.3.3 Procedures*

#### *6.3.3.1 Preliminary testing*

Preliminary visits were undertaken at least 1 wk prior to the three experimental trials. At visit 1, data were collected for height and body mass (Seca 704 r, Seca., Hamburg, Germany), followed by an assessment of  $\dot{V}O_{2\text{peak}}$  and initial coaching of the squat exercise. Subsequent preliminary visits were used to further teach the squat exercise and assess 5-RM for the back-squat. The squat exercise was always preceded by a standardised warm-up (section 3.5.2).

#### *6.3.3.2 Assessment of peak oxygen uptake*

Peak oxygen uptake was measured on the first visit of preliminary testing. Details of the  $\dot{V}O_{2\text{peak}}$  protocol are presented in the General Methods section, 3.4.1.

#### *6.3.3.3 Maximal strength testing*

Maximal strength was predicted from participants' 5-RM performance in the back-squat exercise, and assessed during the final visit of preliminary testing. Details of the maximal strength testing protocol are presented in the General Methods section, 3.4.3.

### *6.3.4 Acute exercise stimulus*

A single exercise stimulus was completed on three occasions, following the preliminary visits. Further details of the single exercise stimulus are presented in the General Methods section, 3.5.

#### *6.3.4.1 Exercise and dietary control*

Details of the exercise and dietary controls prior to the single exercise stimuli are presented in the General Methods section, 3.5.1.

#### *6.3.4.2 Resistance exercise stimulus*

Details of the resistance exercise stimulus are presented in the General Methods section, 3.5.2.

#### *6.3.4.3 Endurance exercise stimulus*

Where randomised, either MIC or HIIC was completed following the resistance exercise stimulus. Details of the endurance exercise stimulus are presented in the General Methods section, 3.5.3.

#### *6.3.4.4 Muscle sampling*

Muscle biopsies were obtained immediately prior to RES and 3 h after completion of RES. All within-trial biopsies were sampled from the same leg, while between-trial biopsies were sampled from alternate legs. Further details of the muscle sampling procedure are presented in the General Methods section, 3.5.4.

#### *6.3.4.5 Blood lactate sampling*

Capillary blood samples were collected from the fingertip at the end of, and following 5 min passive recovery from MIC and HIIC. Details of the blood lactate analysis are presented in the General Methods section, 3.6.1.

### *6.3.5 Analyses*

#### *6.3.5.1 Muscle analysis*

Full details of the muscle analysis are presented in the General Methods section, 3.6.2.

#### *6.3.5.2 Statistical analysis*

Data are presented as mean  $\pm$  SD, with statistical significance set at  $p \leq 0.05$  a priori. Sphericity was assumed if Mauchly's test score returned  $p \geq 0.05$ , with Greenhouse-Geiser adjustments made where appropriate. Blood [La] and HR measures were analysed using a condition (RES + MIC vs. RES + HIIC) by time-point (immediately post- and 5 min post-END) repeated measures ANOVA. The difference in the phosphorylation profile of kinases between-trials was analysed using a one-way ANOVA with repeated measures, comparing condition (RES vs. RES + MIC vs. RES + HIIC) for the fold-change response in phosphorylation from rest to 3 h post-RES. Significant main effects were further investigated using LSD post-hoc, pair-wise comparisons. All data analysis was performed using statistical software (IBM SPSS 22 for Windows., New York, USA).

## 6.4 Results

### 6.4.1 Physiological response

Blood lactate concentration was greater in the HIIC condition compared with MIC following the endurance exercise stimulus (Table 6.1), with a condition ( $F_{[1,7]} = 8.264$ ,  $p = 0.024$ ), time ( $F_{[1,7]} = 14.170$ ,  $p = 0.007$ ), and interaction effect observed ( $F_{[1,7]} = 7.608$ ,  $p = 0.028$ ). The heart rate response during the different endurance exercise stimuli are listed in Table 6.1. Maximum heart rate was greater in the HIIC condition ( $p = 0.001$ ), while average HR across the work- and duration-matched protocols was not different ( $p > 0.05$ ). The time spent in heart rate zones did not differ between the HIIC and MIC conditions ( $p > 0.05$ ; Table 6.2).

**Table 6.1.** Physiological response to the MIC and work-matched HIIC protocols.

Condition	End [La] (mmol·L <sup>-1</sup> )	End + 5 min [La] (mmol·L <sup>-1</sup> )	Max. HR (%max)	Av. HR (%max)
MIC	2.49 ± 1.41	2.04 ± 0.82	91.8 ± 4.3	85.1 ± 4.1
HIIC	6.08 ± 3.83	4.46 ± 2.66	96.9 ± 3.1	85.9 ± 3.1

**Note:** Values presented as mean ± SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling; [La] = blood lactate concentration; HR = heart rate; max. = maximum; av. = average.

**Table 6.2.** Training load quantification of the MIC and work-matched HIIC protocols.

Condition	Zone 1 (%)	Zone 2 (%)	Zone 3 (%)	Zone 4 (%)	Zone 5 (%)
MIC	1.1 ± 2.1	20.8 ± 19.6	36.7 ± 21.4	30.2 ± 23.9	11.2 ± 25.2
HIIC	0.9 ± 1.2	24.8 ± 16.3	21.0 ± 5.8	31.9 ± 4.5	21.4 ± 17.0

**Note:** Values presented as mean ± SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling; zone (%) = % of session in specified heart rate zone.

## 6.4.2 Signalling response

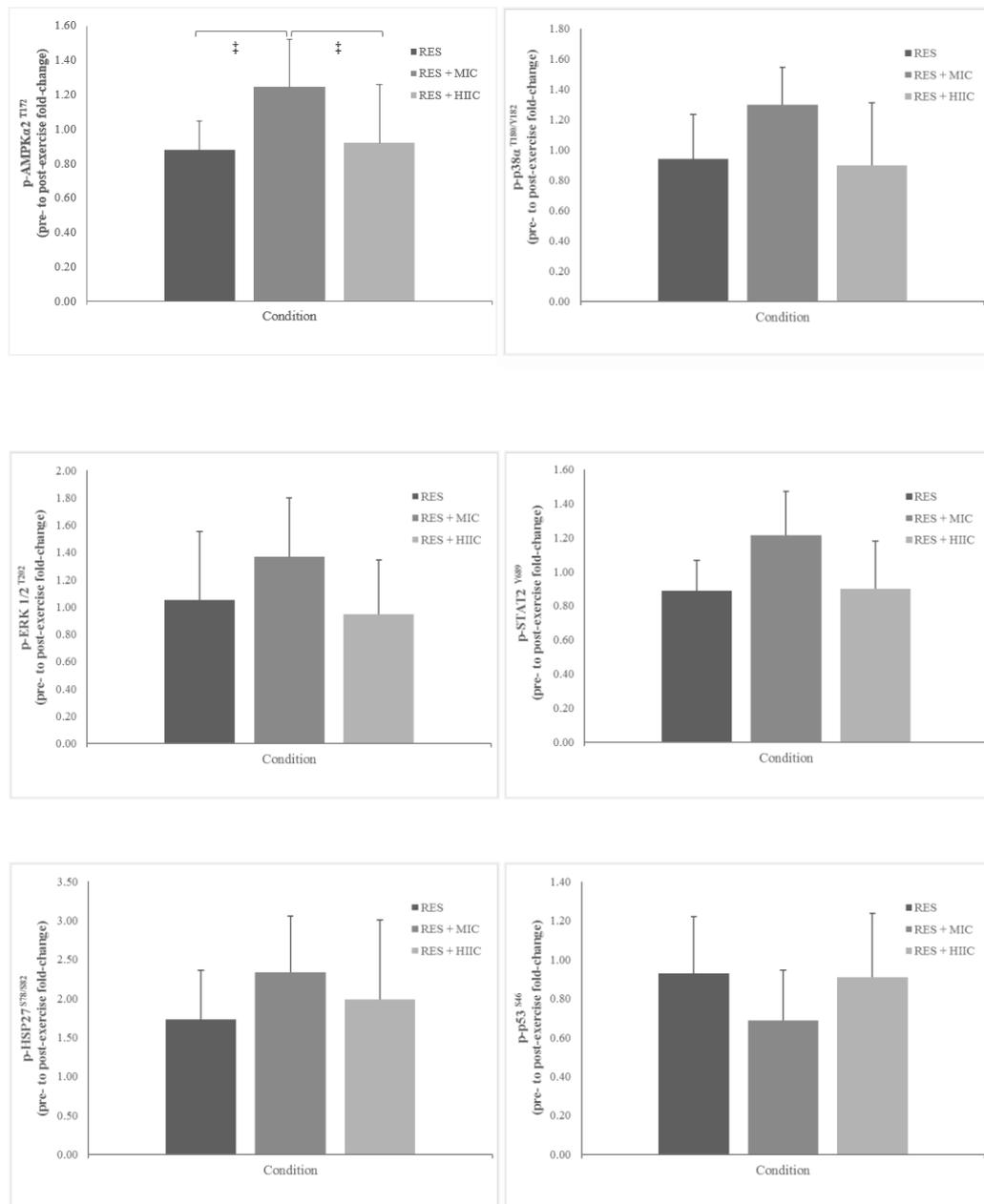
### 6.4.2.1 AMPK pathway

The signalling response of the protein kinases associated with the endurance pathway are presented in Figure 6.1. In the RES + MIC condition, the phosphorylation of p38 $\alpha$ <sup>T180/Y182</sup> was greater at 3 h post-RES, compared with rest ( $1.30 \pm 0.25$  fold-change). This was not true of the RES + HIIC ( $0.90 \pm 0.41$  fold-change) or the RES ( $0.94 \pm 0.29$  fold-change) conditions. This greater fold-change in the RES + MIC condition was not significant ( $p > 0.05$ ). A similar trend was observed in the phosphorylation of AMPK $\alpha$ 2<sup>T182</sup>, with the greatest fold-change increase in RES + MIC ( $1.24 \pm 0.28$ ) in comparison to RES + HIIC ( $0.92 \pm 0.34$ ) and RES ( $0.88 \pm 0.17$ ). There was a main effect of condition for the phosphorylation of AMPK $\alpha$ 2<sup>T182</sup> ( $F_{[2,14]} = 4.046$ ,  $p = 0.041$ ), with a greater response in RES + MIC, relative to both RES + HIIC ( $p = 0.026$ ) and RES ( $p = 0.046$ ).

The phosphorylation of both extracellular signal-regulated kinase (ERK) 1/2<sup>T102</sup> and signal transducer and activator of transcription 2 (STAT2)<sup>Y689</sup> displayed a similar trend between conditions, with the greatest fold-change in the RES + MIC condition ( $1.37 \pm 0.43$  and  $1.21 \pm 0.26$ , respectively). ERK 1/2<sup>T102</sup> phosphorylation was reduced post-exercise in the RES + HIIC trial ( $0.95 \pm 0.40$  fold-change), while displaying a small increase in the RES condition ( $1.05 \pm 0.50$  fold-change). STAT2<sup>Y689</sup> phosphorylation was reduced in both the RES + HIIC and RES conditions ( $0.90 \pm 0.28$  and  $0.89 \pm 0.18$  fold-change, respectively). The differential response between conditions was not significant ( $p > 0.05$ ).

Heat shock protein 27 (HSP27)<sup>S78/S82</sup> phosphorylation was increased post-exercise for all conditions, with a greater fold-change response in the RES + MIC trial ( $2.34 \pm 0.72$ ) compared with the RES + HIIC ( $1.99 \pm 1.02$ ) and RES conditions ( $1.73 \pm 0.63$ ). Conversely, tumour protein p53 (p53)<sup>S46</sup> phosphorylation was reduced post-exercise in all conditions, with the RES + MIC condition displaying the greatest reduction in activation status ( $0.69 \pm 0.26$ ) in comparison to the RES + HIIC ( $0.91 \pm 0.33$ ) and RES trials ( $0.93 \pm$

0.29). There was no main effect for condition for either HSP27<sup>S78/S82</sup> or p53<sup>S46</sup> phosphorylation ( $p > 0.05$ ).

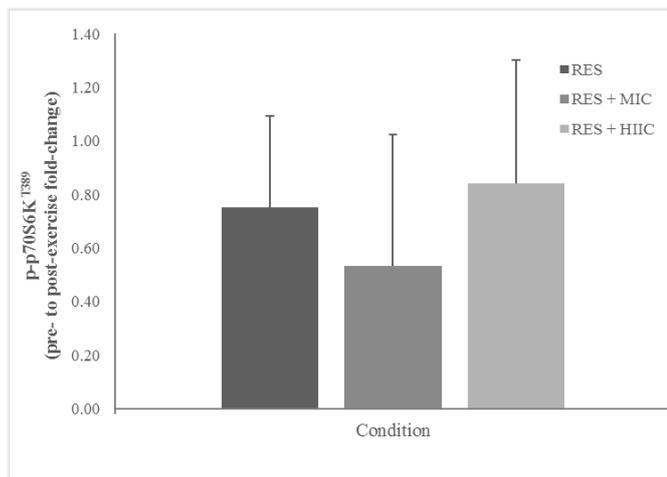
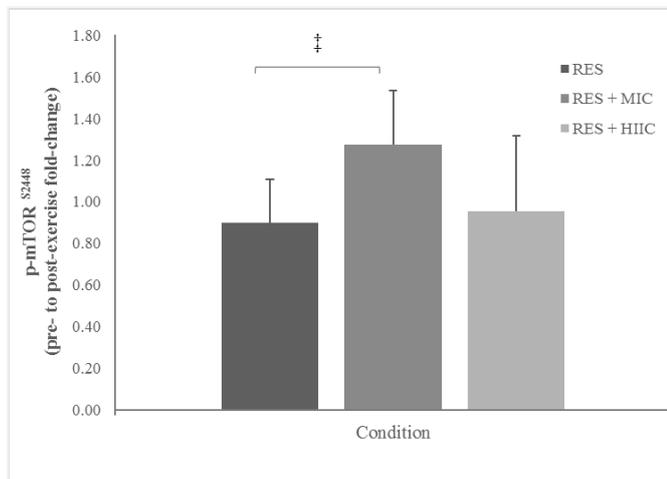
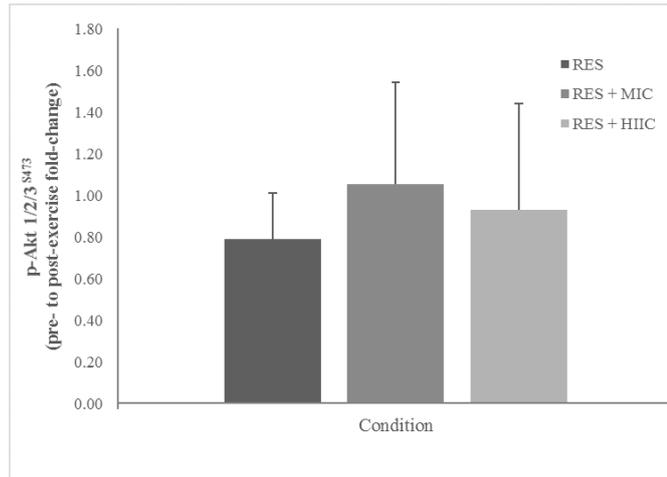


**Figure 6.1.** Mean response in phosphorylation of the AMPK signalling pathway in the RES, RES + MIC, and RES + HIIC conditions. ‡, significantly different from RES + MIC condition ( $p < 0.05$ ). Values presented as mean  $\pm$  SD.

#### 6.4.2.2 mTOR pathway

The signalling response of the protein kinases associated with the strength pathway are presented in Figure 6.2. The phosphorylation of mTOR<sup>S2448</sup> was greatest in the RES + MIC condition ( $1.27 \pm 0.26$  fold-change), with reduced activation status post-exercise in the RES + HIIC and RES conditions ( $0.95 \pm 0.36$  and  $0.90 \pm 0.21$  fold-change, respectively). There was a main effect of condition for the phosphorylation of mTOR<sup>S2448</sup> ( $F_{[2,14]} = 3.963$ ,  $p = 0.043$ ), with a greater response in the RES + MIC relative to RES condition ( $p = 0.033$ ).

A similar trend was observed in the phosphorylation of Akt1/2/3<sup>S473</sup>, with a greater post-exercise phosphorylation in the RES + MIC ( $1.05 \pm 0.49$  fold-change) trial compared with that of the fold-change response in the RES + HIIC ( $0.93 \pm 0.51$ ) and RES ( $0.79 \pm 0.22$ ) conditions. Conversely, this response was greatest in the RES + HIIC condition ( $0.84 \pm 0.46$  fold-change) for the phosphorylation of p70S6K<sup>T389</sup>, with reductions in post-exercise fold-change greater in the RES ( $0.75 \pm 0.34$ ) and RES + MIC trials ( $0.53 \pm 0.49$ ). There was no main effect for condition for either Akt1/2/3<sup>S473</sup> or p70S6K<sup>T389</sup> phosphorylation ( $p > 0.05$ ).



**Figure 6.2.** Mean response in phosphorylation of the mTOR signalling pathway in the RES, RES + MIC, and RES + HIIC conditions. ‡, significantly different from RES + MIC condition ( $p < 0.05$ ). Values presented as mean  $\pm$  SD.

## 6.5 Discussion

This work aimed to observe whether a concurrent exercise stimulus (independent of intensity) would result in an inhibition of anabolic signalling proteins, relative to a strength stimuli performed in isolation. Further, this work aimed to examine the idea that intensity of the endurance stimuli would affect the presence or magnitude of a molecular interference effect. These aims were in the context of an endurance-trained, but strength-training naive phenotype. The major findings were that 1) despite differential AMPK and mTOR signalling between conditions, this was not suggestive of an interference effect i.e. an antagonistic relationship; 2) despite differential activation status of the AMPK and mTOR signalling cascades, this did not support the idea of an intensity-dependent regulation of AMPK; 3) the concurrent exercise stimuli resulted in a generally smaller response in the phosphorylation of all the protein targets observed.

The majority of research aiming to observe a molecular interference has focused on studying concurrent stimuli *vs.* resistance stimuli in isolation amongst recreationally active cohorts, using the activation status of the mTOR signalling cascade as a reference for the early response of the anabolic machinery (Apro et al., 2013, Fernandez-Gonzalo et al., 2013, Lundberg et al., 2012). This line of enquiry has ultimately provided a lack of support for the theory of a molecular interference effect. Whilst some investigations have reported no difference in the activation status of mTOR and AMPK signalling networks following the manipulation of exercise stimuli (Apro et al., 2013, Jones et al., 2015), there are also numerous data displaying trial effects inconsistent with the premise of a molecular interference effect (Apro et al., 2015, Lundberg et al., 2012, Pugh et al., 2015).

Apro et al. (2015) reported elevated AMPK phosphorylation in the concurrent exercise condition, relative to a resistance only stimulus, without a subsequent inhibition of mTOR activation status in the concurrent exercise condition. There are data from Lundberg et al. (2012) displaying elevated mTOR and p70S6K activation status following endurance-

resistance, compared with resistance only exercise. Similarly, elevated mTOR phosphorylation was observed in the concurrent condition, relative to resistance exercise (Pugh et al., 2015). Collectively, this existing literature counter the existence of a molecular interference by failing to demonstrate an antagonistic relationship between mTOR and AMPK signalling networks and elevated mTOR network activity with the removal of endurance stimuli from concurrent exercise. The findings of this work are consistent, in part, with the aforementioned literature. Firstly, the elevated phosphorylation of AMPK in the RES + MIC vs. RES conditions was not observed in conjunction with an inhibition of mTOR activation status in the RES + MIC condition. Secondly, mTOR phosphorylation was upregulated in the RES + MIC vs. RES conditions. Hence, while the addition of an endurance stimulus in the RES + MIC condition was sufficient to upregulate AMPK activation status, relative to resistance exercise in isolation, this was consistent with the trend for mTOR phosphorylation and counters the principle of a molecular interference effect.

Exercise intensity is a key training variable, and if endurance activity is purported to be antagonistic to early growth responses, it would seem logical that a greater endurance exercise intensity might exacerbate the issue. This logic has received some investigation in the context of recreationally-active individuals (Fyfe et al., 2016b). Authors compared resistance exercise in isolation with work and duration-matched concurrent models, incorporating either moderate or high intensity exercise. AMPK phosphorylation was similarly upregulated across all conditions, while mTOR activation status was only elevated in the high intensity concurrent condition. In addition, despite p70S6K activation status increasing following each of the conditions, there was no difference in the response between conditions (Fyfe et al., 2016b). Hence, from these data, it would seem that concurrent exercise with a high intensity endurance component would be preferential compared to resistance exercise in isolation or a moderate-intensity concurrent stimulus, in creating an anabolic environment. Elevated mTOR signalling has previously been observed

following intensive aerobic exercise in untrained individuals (Mascher et al., 2011), in agreement with the premise of a generic molecular footprint following exercise that the individual is unaccustomed to (Coffey et al., 2006). If the recreationally active cohort from the work of Fyfe et al. (2016b) were less accustomed to high intensity endurance activity, relative to moderate intensity stimuli, this might help to explain the observed mTOR phosphorylation responses. Further, the data fail to support the intensity-dependent regulation of AMPK, which has also been refuted in a running exercise model (Bartlett et al., 2012).

The work of Fyfe et al. (2016a) offers data consistent with the key findings of this study, in respect to a lack of evidence for both an interference effect and the intensity-dependent regulation of AMPK. However, an inconsistency was that the greatest mTOR activation status was observed with a higher intensity of endurance exercise; the data from this study suggest that the moderate intensity concurrent stimulus would be preferential. Regardless of which concurrent stimulus provided a superior stimulus of the anabolic machinery, both contradict the theory of an interference effect, in which the resistance only stimulus would afford an enhanced strength response. Whilst unexpected, this finding is not unique; others have reported augmented mTOR activation status following concurrent exercise *vs.* resistance exercise in isolation (Lundberg et al., 2012).

The observation of the AMPK and mTOR signalling networks in the early post-exercise period has been a popular approach in efforts to better understand the interference effect (Apro et al., 2013, Fernandez-Gonzalo et al., 2013, Lundberg et al., 2012). Activity of the mTOR signalling cascade is often observed, with the inference that it provides an apt proxy for the subsequent processes of MPS and hypertrophy. Indeed, Cuthbertson et al. (2006) demonstrated increased phosphorylation of targets within the mTOR network following a repeated stepping protocol, with a concomitant but delayed response in MPS. The direct observation of protein accretion is rare within the concurrent exercise literature (Apro et al., 2015, Carrithers et al., 2007). Carrithers et al. (2007) reported no difference in

MPS between resistance only and concurrent exercise conditions. This finding was consistent with the work of Apro et al. (2015), despite a differential response in downstream targets of the mTOR signalling cascade between the resistance only and concurrent condition. This observation is not unique, with a discord between MPS and mTOR signalling previously reported in a protein feeding model (Atherton et al., 2010). Such findings highlight the potential incongruity of using anabolic signalling as a proxy for MPS. Further, while adopting the methodological complexities of measuring MPS should be commended, the magnitude of protein accretion still does not provide assurance as to the potential for training adaptation (Mitchell et al., 2014).

Numerous investigations concerning the acute interference effect have utilised an endurance followed by resistance exercise model as a concurrent stimulus (Apro et al., 2015, Carrithers et al., 2007, Fyfe et al., 2016b, Lundberg et al., 2012). As discussed, this is with a view to better understand the potential inhibitory effect of endurance exercise on the anabolic stimulus presented. The meta-analysis presented in Chapter 5 indicates a beneficial effect of a resistance followed by endurance exercise order for lower-body strength adaptation across a short-term concurrent training programme. This would suggest that such an exercise sequence would be an appropriate model to examine the interference effect; an exercise sequence which is beneficial for strength adaptation. If this model were to provide a greater acute strength stimulus, this would constitute a more ecologically valid vehicle to investigate the inhibitory effects of concurrent exercise, and is the model adopted in this work.

The exercise stimuli used in this study resulted in a generally small response in the phosphorylation of protein targets observed, compared with some of the existing literature. This study observed peak magnitudes of ~1.5-fold increase in the phosphorylation of targets, with the exception of HSP27 (~2.5-fold). Previous research reported substantial perturbations to downstream targets of the mTOR signalling network in response to exercise; a 14-fold (Apro et al., 2013), 12-fold (Apro et al., 2015), and 16-fold (Wang et al.,

2011) increase in p70S6K activation status at residue Thr<sup>389</sup>. However, there are data which highlights the variable nature of quantifying signalling responses associated with the mTOR and AMPK signalling cascades, reflected by observations of much smaller magnitudes of upregulation or even decreased activation status in protein targets; a 1.8-fold increase in p70S6K<sup>T389</sup> activation status (Lundberg et al., 2012); a decrease in Akt<sup>S473</sup> phosphorylation (Pugh et al., 2015); a decrease in AMPK<sup>T172</sup> phosphorylation (Apro et al., 2013). Hence, there is a lack of agreement in the activation status of key targets within the aforementioned signalling cascades.

Where discrepancies do present in the magnitude of signalling responses between this work and existing literature, this might be explained by the numerous methodological variables encountered with a concurrent exercise model. Training status is an important non-training variable, with training history reported to attenuate the molecular response in the early post-exercise period (Coffey et al., 2006). Given the endurance training status of the participants in this study, a muted response in the phosphorylation of targets associated with the AMPK signalling cascade could have been anticipated. However, the response in anabolic signalling was somewhat more surprising, with the phosphorylation of AMPK and downstream targets comparable to that of the growth-associated signalling cascade. Nutrition is another non-training variable capable of modulating the molecular response to an exercise stimulus, with the provision of amino acids inducing a stimulatory effect on the growth-associated signalling network (Deldicque et al., 2005). As such, great effort was employed to control diet in this work. Some of the previous literature failed to employ such rigour concerning dietary intake prior to the exercise stimulus, instead asking participants to record and duplicate food intake (Apro et al., 2015, Wang et al., 2011). Much of the research in the literature has utilised a fasted model of exercise (Apro et al., 2013, Coffey et al., 2009a, Coffey et al., 2009b). In line with other models (Fyfe et al., 2016b, Jones et al., 2015, Lundberg et al., 2012), this work provided a small amount of protein prior to exercise (0.1 g·kg<sup>-1</sup>·d<sup>-1</sup> protein). This inconsistency in method could increase the magnitude of

signalling of the mTOR network, and hence fail to explain the smaller magnitude of change observed compared to some of the data in the literature (Apro et al., 2015, Apro et al., 2013, Wang et al., 2011).

Other training programme variables might also act to modulate the adaptive response to exercise stimuli (Fyfe and Loenneke, 2018). For example, an exercise mode of running vs. cycling (Wilson et al., 2012), an increase in endurance session frequency (Jones et al., 2013), and reduced recovery between exercise modes (Robineau et al., 2016) have been reported to increase the likelihood of interference. In the current study, the decision was made to better represent the applied scenario and the nature of athletic training. As such, this work used a strength exercise model to better mimic the real-life training setting. The use of leg press machine or dynamometer is common place within the body of literature (Carrithers et al., 2007, Coffey et al., 2009a, Pugh et al., 2015), which possess questionable ecological validity. Instead, the current work employed a back-squat exercise to stimulate the *quadriceps*. This exercise has been reported to activate the *vastus lateralis* similarly compared with alternate resistance training exercises (Ebben et al., 2009). Therefore, while inconsistent with some of the previous literature, the inclusion of this exercise should be unlikely to explain the moderate activity of the molecular targets measured.

In conclusion, these data fail to support an acute molecular interference effect in a trained endurance cohort. The data also fail to support the intensity-dependent regulation of AMPK, when comparing a work and duration-matched moderate and high intensity concurrent exercise stimulus. Finally, the findings add to the growing body of literature, suggestive of mTORC1 and AMPK to be poor correlates to investigate the mechanism explaining concurrent interference, particularly in an acute paradigm.

## 6.6 Summary

This study investigated whether the intensity of the endurance stimulus affects the activation status of signalling proteins associated with the mTOR and AMPK networks. Further, the study examined whether a molecular interference was observed amongst trained endurance cyclists. The primary finding was a lack of support for an acute molecular interference effect in a trained endurance cohort. Further, in an acute context, the data failed to support the intensity-dependent regulation of AMPK, nor differential activation of the anabolic machinery with the manipulation of endurance exercise intensity. These data suggest that endurance athletes might not need be concerned with the intensity of their endurance session (moderate *vs.* high intensity) affecting their strength adaptation, when the two exercise modes are performed in close proximity to one another. This work addresses a specific aim of the thesis, by assessing whether acute interference is evident in an endurance-trained cycling cohort, and whether endurance exercise intensity plays a role in mitigating the potential detrimental effect. Caution should be exercised however, as these findings are specific to an acute context. This question should be examined across a training period, in order to provide greater confidence in the longer-term effects. The subsequent chapter will address this question specifically.

**7. THE ROLE OF ENDURANCE  
EXERCISE INTENSITY DURING  
SHORT-TERM CONCURRENT  
TRAINING**

## 7.1 Abstract

This study examined whether the intensity of endurance training stimuli modifies the adaptation in lower-body strength and cycling-specific endurance following concurrent training. Further, whether the acute molecular response to concurrent exercise stimuli is affected by training status i.e. pre vs. post, or differentially affected in relation to the endurance intensity prescribed throughout the training intervention. These questions were answered using trained endurance cyclists, which were naïve to strength training at the onset of the research. Using a parallel group design, participants were randomised to either resistance exercise followed by moderate intensity cycling (RES + MIC), or resistance exercise followed by work- and duration-matched high intensity interval cycling (RES + HIIC), across an 8 wk training programme. Two group-specific sessions were performed per week, separated by  $\geq 48$  h between sessions. A single group-specific exercise stimulus was completed at least 1 wk before and within 5 d of completing the training programme, to assess phosphorylation of protein kinases associated with the mTOR and AMPK signalling pathways. A main effect for time was observed for each of the maximal strength exercises; the back-squat, split-squat, and calf-raise ( $p < 0.001$ ), while no time effects were observed across the measures of 5 min TT performance, power at  $2 \text{ mmol}\cdot\text{L}^{-1}$ , power at  $4 \text{ mmol}\cdot\text{L}^{-1}$ , or  $\dot{V}\text{O}_{2\text{peak}}$  ( $p > 0.05$ ). No group effects were observed for any of the aforementioned outcomes ( $p > 0.05$ ). Lastly, there were no main effects for time or group for the phosphorylation of protein kinases in response to the single concurrent exercise stimulus conducted pre and post-training intervention ( $p > 0.05$ ). These results indicate that the intensity of endurance activity (as part of concurrent training stimuli) had no effect on performance outcomes, following short-term concurrent training. Importantly, this was in the context of improvements in strength and power parameters. Further, the acute molecular response to a concurrent exercise stimulus was comparable before and after the training intervention, suggesting that training status had no effect on the molecular responses assessed, and not differentially activated by the intensity of an endurance stimulus.

## 7.2 Introduction

A concurrent training model has long been associated with an interference effect, whereby strength adaptation is inhibited when practicing concurrent training *vs.* strength training in isolation (Hickson, 1980). Establishing effective training methods within a concurrent exercise paradigm requires practitioners to manipulate acute training variables, in order to elicit targeted adaptations. The effectiveness of the training programme therefore rests on the intricacies of manipulating exercise frequency, intensity, sequence, duration and mode. Given the necessity for numerous elite sporting populations to develop strength and aerobic capacity simultaneously, optimising training methods with the manipulation of acute training variables is crucial.

Investigations to identify mechanisms underpinning the potential interference effect followed the seminal work of Hickson (1980). Residual fatigue was initially theorised to provide a possible explanation for the interference effect, owing to the decline in strength adaptation occurring in the latter stages of the training programme in the concurrent group relative to the resistance training group, with a further suggestion that biochemical processes of adaptation might prove a mechanistic reason for these observations (Hickson, 1980). Subsequent work has offered additional possibilities to explain the interference effect, such as glycogen depletion acting to reduce the quality of strength training following endurance stimuli (Jacobs et al., 1981), or indeed, acting to attenuate anabolic signalling (Creer et al., 2005). Further, a decline in the neural input to the muscle and peripheral contractile mechanisms subsequent to endurance stimuli (Lepers et al., 2000), or antagonistic processes at the molecular level which inhibit the potential for strength adaptation (Fyfe et al., 2014, Nader, 2006).

Given that the divergence in strength adaptation between groups occurred from 5-10 wk in the work of Hickson (1980), it would seem appropriate to address such questions over a similar timeframe (Hamilton and Philp, 2013). Aside from the examination of an

interference effect, it is of great benefit to athletes following concurrent training practice, to better understand the role of acute training variables in eliciting strength adaptation. The vast majority of chronic investigations exploring approaches to mitigate an interference effect have focussed on manipulating exercise sequence of the opposing exercise components (Chtara et al., 2008, Collins and Snow, 1993, McGawley and Andersson, 2013). Additionally, Robineau et al. (2016) observed the variable of frequency, through the manipulation of recovery duration, while few have investigated the effect of manipulating endurance exercise intensity (Fyfe et al., 2016a, Silva et al., 2012).

Exercise intensity is a key training variable. HIIT can offer adaptations consistent, if not superior to that of traditional endurance training, with regards to aerobic capacity (Gormley et al., 2008, Hwang et al., 2011, Wisloff et al., 2007) and is effective in eliciting endurance adaptations in well-trained cohorts (Skovereng et al., 2018). If endurance activity is purported to be antagonistic to strength adaptation, it would seem logical that a greater endurance exercise intensity would exacerbate the issue. This could be particularly relevant given that AMPK phosphorylation is greater following higher intensity (85%  $\dot{V}O_{2peak}$ ) vs. lower intensity cycling exercise (35%  $\dot{V}O_{2peak}$ ), supporting the intensity-dependent regulation of AMPK (Rose et al., 2009). However, the relevance of AMPK activation status should be treated with caution, as there are data to suggest that AMPK phosphorylation does not inhibit acute growth-related responses after subsequent strength stimuli (Apro et al., 2015).

Experimentally, just two groups have explored the question of endurance exercise intensity in the context of concurrent training, specific to recreationally active individuals (Fyfe et al., 2016a, Silva et al., 2012). Silva et al. (2012) reported no interference effect, nor any group differences across strength and endurance outcomes following the manipulation of endurance exercise intensity. In contrast, Fyfe et al. (2016a) did report an interference effect across lower-body strength and power measures, but similarly failed to observe any effect of endurance exercise intensity following a concurrent training intervention.

Regardless of whether an interference effect does exist across a training period, it is likely to be of greater importance to the athlete to understand whether a lower or higher intensity endurance component might be advantageous to performance outcomes following concurrent training.

The literature supports the inclusion of lower-body strength training for endurance cycling cohorts (Rønnestad et al., 2010, Rønnestad et al., 2011, Rønnestad et al., 2015). Therefore, a trained endurance cohort should prove a suitable population to investigate the role of endurance exercise intensity within a concurrent training programme. Researchers in the field have proposed that training status of the individual will likely have an important role on the adaptive response to a concurrent training intervention. Specifically, these authors suggest that training status might modify the early molecular signalling responses to exercise, with an attenuated response amongst trained phenotypes and a generic molecular footprint response in untrained cohorts (Coffey and Hawley, 2016). Observing the early molecular response to a concurrent exercise stimulus pre and post-training intervention might help to substantiate these suggestions.

The purpose of this study was twofold. Firstly, to examine whether the intensity of endurance stimuli throughout a concurrent training block affected performance outcomes. Secondly, to observe whether the acute molecular response to concurrent exercise stimuli is affected by training status i.e. pre vs. post, or differentially affected in relation to the endurance intensity prescribed throughout the training intervention. These questions were to be answered within the context of trained endurance cyclists, which were naïve to strength training at the onset of the research. This chapter will therefore address a specific aim of the thesis, by assessing whether endurance exercise intensity modifies the adaptation in lower-body strength and cycling-specific endurance following concurrent training, and whether the acute response to a concurrent stimulus is modified in a concurrent-trained state.

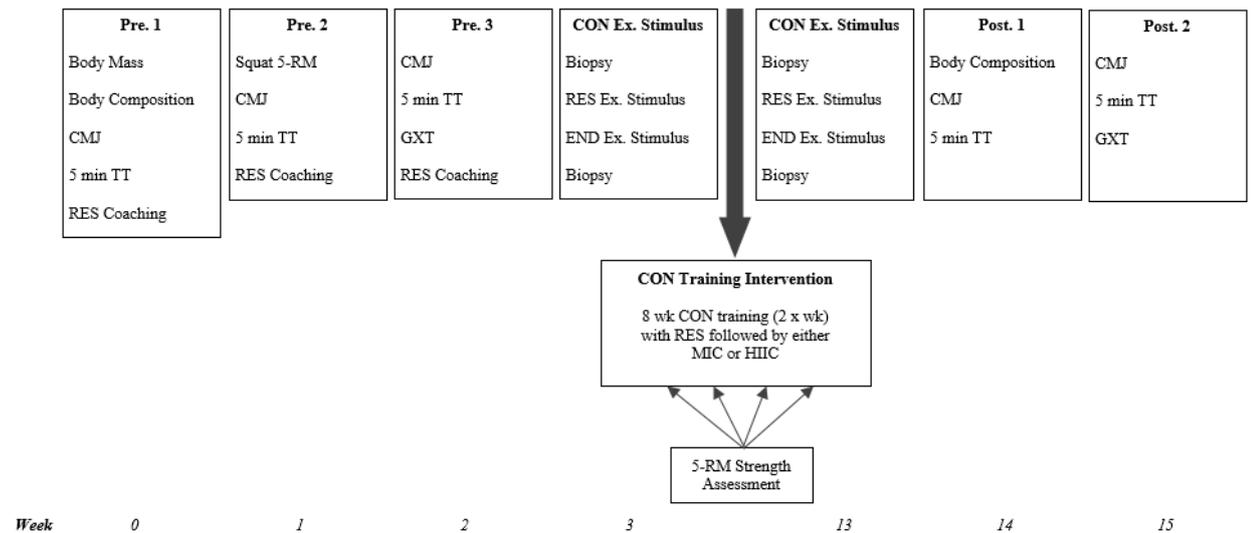
## 7.3 Methods

### 7.3.1 Design

The study utilised a repeated-measures, parallel group design. Following three preliminary trials for familiarisation to procedures and collection of baseline data, participants were ranked on predicted 1-RM back-squat performance. Participants were subsequently randomised, in a stratified fashion, to either, 1) resistance exercise followed by moderate intensity cycling (RES + MIC), or 2) resistance exercise followed by work- and duration-matched high intensity interval cycling (RES + HIIC). Participants then completed an 8 wk training programme, with two group-specific sessions performed per week, separated by  $\geq 48$  h between sessions. Maximal strength was assessed at each quarter of the training programme, while other performance outcomes were repeated post-intervention. A single group-specific exercise stimulus was completed at least 1 wk before and within 5 d of completing the training programme, to assess phosphorylation of protein kinases associated with the mTORC1 and AMPK signalling pathways.

Preliminary visits were used to collect descriptive data; height and body mass, provide familiarisation to and collect baseline data for performance outcomes; aerobic thresholds, back-squat 5-RM, CMJ, 5 min TT, and body composition (each described subsequently). The remainder of the preliminary visits were used to coach the lower-body strength exercises included in the training programme; split-squat and calf-raises (Figure 7.1).  $\dot{V}O_{2\text{peak}}$  and 5-RM data were used to prescribe relative exercise intensities for the single concurrent exercise stimulus and training intervention. The single concurrent exercise stimulus required participants to complete RES (6 x 8 back-squat repetitions at 80% predicted 1-RM) followed by either MIC (continuous 40 min cycling at 65%  $\dot{V}O_{2\text{peak}}$ ) or HIIC (40 min cycling with 3 min intervals of 85 and 45%  $\dot{V}O_{2\text{peak}}$ ). The cycling protocols are depicted in the General Methods section, 3.5.3. Muscle biopsies were collected at rest and 3 h post-RES. The same intra-session order i.e. resistance followed by

endurance, was used throughout the training programme, with session load periodised across the 8 wk duration (Table 7.1). The intra-session order used has been reported to be preferential for lower-body strength adaptation across a short-term concurrent training programme (Eddens et al., 2018).



**Figure 7.1.** Study schematic. CMJ = counter-movement jump; TT = time trial; RES = resistance exercise; END = endurance exercise; CON = concurrent; Ex. = exercise; wk = week; MIC = moderate intensity cycling; HIIC = high intensity interval cycling; 5-RM = 5-repetition maximum; GXT = graded exercise test.

### 7.3.2 Participants

Fourteen male, trained endurance cyclists volunteered to take part in the study; however, one participant withdrew due to circumstances unrelated to the study. Thirteen participants (age  $30 \pm 6$  years; height  $179 \pm 4$  cm; body mass  $71.8 \pm 7.4$  kg;  $\dot{V}O_{2peak}$   $55.9 \pm 7.0$  ml·kg<sup>-1</sup>·min<sup>-1</sup>; back-squat 1-RM  $107.9 \pm 31.2$  kg) completed the study. Participants had no resistance training history for  $\geq 6$  months prior to enrolment. Additional information relating to the ethical approval of the study and its participants is presented in the sections, 3.2 and 3.3, respectively.

### 7.3.3 Preliminary procedures

#### 7.3.3.1 Preliminary testing

Preliminary visits were undertaken at least 1 wk prior to the single concurrent exercise stimulus. At visit 1, data were collected for height and body mass (Seca 704 r, Seca., Hamburg, Germany), followed by an assessment of body composition. Maximal strength was assessed at visit 2, while data were collected for CMJ, 5 min TT, and aerobic profile at visit 3. These preliminary visits were also used to familiarise participants with the CMJ and 5 min TT performance tests, in addition to coaching of the lower-body strength exercises to the strength-trained naïve cohort.

#### 7.3.3.2 Graded exercise test

Details of the graded exercise test are presented in the General Methods sections, 3.4.1. and 3.6.1.

#### 7.3.3.3 Assessment of peak oxygen uptake

After completion of the graded exercise test, a 15 min period of rest was initiated before completing the aerobic profile assessment. During this period, participants were free to drink water *ad libitum* and move around the laboratory. The  $\dot{V}O_{2\text{peak}}$  protocol was then completed. Details of the  $\dot{V}O_{2\text{peak}}$  protocol are presented in section, 3.4.1.

#### 7.3.3.4 Body composition

Height (stretch stature), mass and skinfolds were collected in accordance with the standard procedures recommended by the International Society for the Advancement of Kinanthropometry (ISAK) (Marfell-Jones et al, 2006). Measures were recorded for eight skinfold thicknesses (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, anterior thigh, and medial calf), using Harpenden skinfold callipers (Baty International., West Sussex, UK). Each site was measured in duplicate, with a third collected if the technical error of measurement (TEM) threshold advised by ISAK was breached for a given

site. The equation adapted from (Withers et al., 1987) was used to estimate percent body fat ( $Eq. (2) BF\% = 495/(1.0988-0.0004 * \Sigma 7)-450$ ) and calf girth was also measured. This enabled measures for sum of 7 skinfolds ( $\Sigma 7$ ), body density, body fat percentage (BF%), fat mass, and fat-free mass. All assessments were conducted by the same certified anthropometrist, with a mean TEM of 1.95% across the respective measures.

#### *7.3.3.5 Counter-movement jump*

The CMJ protocol was always preceded by a standardised 5 min warm-up at an intensity of 50%  $\dot{V}O_{2peak}$  on an electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA), followed by a 5 min standardised dynamic warm-up consisting of heel to toe walking, goblet squats, squat jumps, and stiff-leg jumps. Details of the CMJ protocol are presented in the General Methods section, 3.4.2.

#### *7.3.3.6 5 min time trial*

Following a standardised 5 min warm-up at an intensity of 50%  $\dot{V}O_{2peak}$ , participants completed a 5 min TT using an electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA). The assessment required participants to complete the maximum distance possible in a 5 min period. The trial started with the ergometer set in the lowest possible gear ratio, whereby after a 3 s count-down, the participant was responsible for manipulating gearing to a desired level. Feedback of performance data was withheld, except time elapsed, which was communicated only at the half way point (2.5 min) and participants were permitted to change gears as and when they felt necessary. Heart rate was continually recorded throughout each trial, using wireless telemetry (T31 transmitter, Polar Electro Ltd., Kempele, Finland) and participants were cooled with an electric fan on a standardised setting.

#### *7.3.3.7 Maximal strength testing*

Maximal strength was predicted from participants' 5-RM performance in the three lower-body exercises; back-squat, split-squat, and calf-raise. Details of the maximal strength testing protocol are presented in the General Methods section, 3.4.3.

#### *7.3.4 Single concurrent exercise stimulus*

##### *7.3.4.1 Exercise and dietary control*

Details of the exercise and dietary control for the single concurrent exercise stimulus are presented in the General Methods section, 3.5.1.

##### *7.3.4.2 Resistance exercise stimulus*

Details of the resistance exercise stimulus are presented in the General Methods section, 3.5.2.

##### *7.3.4.3 Endurance exercise stimulus*

Details of the endurance exercise stimulus are presented in the General Methods section, 3.5.3.

##### *7.3.4.4 Muscle sampling*

Details of the muscle sampling procedures are presented in the General Methods section, 3.5.4.

##### *7.3.4.5 Blood sampling*

Capillary blood samples were collected from the fingertip at the end of, and following 5 min passive recovery from MIC or HIIC during the single concurrent exercise stimulus, before the training intervention commenced. Details of the blood lactate analysis are presented in the General Methods section, 3.6.1.

### 7.3.5 Training intervention

Participants began the training intervention  $\geq 1$  wk following the initial single concurrent exercise stimulus. The RES stimulus was identical between groups and was always completed first in the session, with MIC or HIIC commencing within 5 min of completing RES. The training intervention was modified to allow for an overload stimulus, by increasing load lifted following intermediary strength assessments, or by increasing the duration of the MIC or HIIC sessions (described subsequently). Participants were required to complete  $\geq 95\%$  of the scheduled training sessions, all of which were to be completed in the laboratory under supervision. A maximum of four participants could be trained in the laboratory at any one time, with the two investigators supervising and providing verbal encouragement to motivate participants to complete the sessions.



**Figure 7.2.** The experimental set up for a supervised training session.

### 7.3.5.1 Resistance training

The resistance training programme was performed twice per week and incorporated three strength exercises; the back-squat, split-squat, and calf-raise. The techniques were conducted in line with standardised procedures (Ebben et al., 2009, Ronnestad et al., 2012) and supervised by a qualified strength and conditioning coach. The squat technique is reported to provide a stimulus of the *vastus lateralis*, comparative to that of alternate lower-body strength exercises (Ebben et al., 2009). This was of paramount importance, owing to the *vastus lateralis* muscle being harvested for insight into the mechanistic response to concurrent exercise at pre and post-training intervention. Further, the three exercises incorporated are reported to improve parameters of strength, jump height, and muscle CSA amongst trained cyclists (Ronnestad et al., 2010, Ronnestad et al., 2017). Each visit started with the same standardised warm-up as completed prior to maximal strength testing (presented in section 3.4.3), followed by two sets of back-squat of increasing load (40 and 60% of predicted 1-RM) and decreasing number of repetitions (10 and 8, respectively). A back-squat, split-squat, calf-raise order was followed and sets were separated by a 3 min rest period. Intermediary assessments of maximal strength were conducted throughout the intervention and session load was modified if maximal strength had increased. The resistance programme was modified from the work of Ronnestad et al. (2010), providing a periodised intervention, containing a hypertrophy and strength stimulus. The resistance programme is presented in full in Table 7.1. The strength and conditioning coach cued the participants to complete the repetitions with maximal intended movement velocity (Behm, 1995).

**Table 7.1.** Detail of the 8 wk resistance training programme.

Phase	Week	Session	Detail
	1	1	5RM assessment
1	1	2	3 sets, 10 reps @ 75% 1RM
1	2	3	3 sets, 6 reps @ 85% 1RM
1	2	4	3 sets, 10 reps @ 75% 1RM
	3	5	5RM assessment
2	3	6	3 sets, 8 reps @ 80% 1RM
2	4	7	3 sets, 5 reps @ 87% 1RM
2	4	8	3 sets, 8 reps @ 80% 1RM
2	5	9	3 sets, 5 reps @ 87% 1RM
2	5	10	3 sets, 8 reps @ 80% 1RM
	6	11	5RM assessment
3	6	12	3 sets, 6 reps @ 85% 1RM
3	7	13	3 sets, 4 reps @ 90% 1RM
3	7	14	3 sets, 6 reps @ 85% 1RM
3	8	15	3 sets, 4 reps @ 90% 1RM
	8	16	5RM assessment

**Note:** 5RM = 5 repetition maximum; 1RM = 1 repetition maximum; reps = repetitions

### 7.3.5.2 Endurance training

Participants completed either MIC or work- and duration-matched HIIC, within 5 min of completing the RES training stimulus. MIC entailed constant load cycling at 65%  $\dot{V}O_{2peak}$ , while HIIC required participants to perform 3 min intervals of 85 and 45%  $\dot{V}O_{2peak}$ , using an electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Seattle,

USA). Both protocols contained a warm-up and cool down and are presented in full in the General Methods section, 3.5.2. Heart rate was recorded throughout each session (Polar A300 transmitter, Polar Electro Ltd., Kempele, Finland), while visual feedback of time elapsed, power output, and pedal cadence were made available for participants. Session duration was modified at week five, to incorporate another set of intervals for the HIIC group, or another 6 min of cycling at 65%  $\dot{V}O_{2peak}$  for the MIC group. Participants were cooled with an electric fan on a standardised setting. Training and performance tests were performed on the same cycling ergometer.

#### *7.3.5.3 Training load quantification*

Laboratory (prescribed) and non-laboratory (non-prescribed) training load was quantified for endurance training completed by all participants. Work performed (external load) during laboratory training visits was matched for the two groups, relative to maximal aerobic capacity. Heart rate, rate of perceived exertion (RPE), and session duration data were collected for both laboratory and non-laboratory endurance training performed across the intervention period. The internal training load was then quantified using the session RPE model (Foster et al., 2001) and by using duration in individual heart rate zones (Halson et al., 2002, Halson and Jeukendrup, 2004), multiplied by the zone weighting factor i.e. 1, 2, 3, 4, or 5 to provide a training impulse score (Halson, 2014). RPE for the session was assessed with Borg's modified CR-10 scale, with the score multiplied by session duration to provide total load. These data were collected with the use of an online training survey sheet ([www.docs.google.com](http://www.docs.google.com)), to assist the participants with logging the duration of the session and the associated RPE. This process was completed within 30 min of training session completion. Further, each participant was provided with a heart rate monitor (Polar A300 transmitter, Polar Electro Ltd., Kempele, Finland), with both laboratory and non-laboratory training session data to be uploaded to the manufacturer's portal ([www.flow.polar.com](http://www.flow.polar.com)). A sync from the participant's watch to the laboratory iPad was conducted at the end of each training session, which ensured that data from that

laboratory session and any external training since the previous laboratory session, was uploaded to the manufacturer's portal. The same device was used to monitor both laboratory and external heart rate responses.

#### *7.3.5.4 Exercise and dietary control*

Participants were deemed fit for training if they could confirm that they were free from medications/vitamin supplementations and had refrained from external training and alcohol for 24 h. Participants were asked to maintain habitual diet and exercise practices throughout the duration of the study. Participants were not permitted to practice external resistance training throughout the course of the study.

### *7.3.6 Analyses*

#### *7.3.6.1 Muscle analysis*

Details of the muscle analysis are presented in the General Methods section, 3.6.2.

#### *7.3.6.2 Statistical analysis*

Data are presented as mean  $\pm$  SD, with statistical significance set at  $p \leq 0.05$  a priori. Sphericity was assumed if Mauchly's test score returned  $p \geq 0.05$ , with Greenhouse-Geiser adjustments made where appropriate. All measures which were repeated at different time points throughout the training intervention i.e. maximal strength, were analysed using a condition (RES + MIC vs. RES + HIIC) by time-point (pre vs. post-intervention) repeated measures ANOVA. Further, single time point measures i.e. training load, were analysed using an independent samples t-test (RES + MIC vs. RES + HIIC). Significant main effects were further investigated using LSD post-hoc, pair-wise comparisons. All data analysis was performed using statistical software (IBM SPSS 22 for Windows., New York, USA).

## 7.4 Results

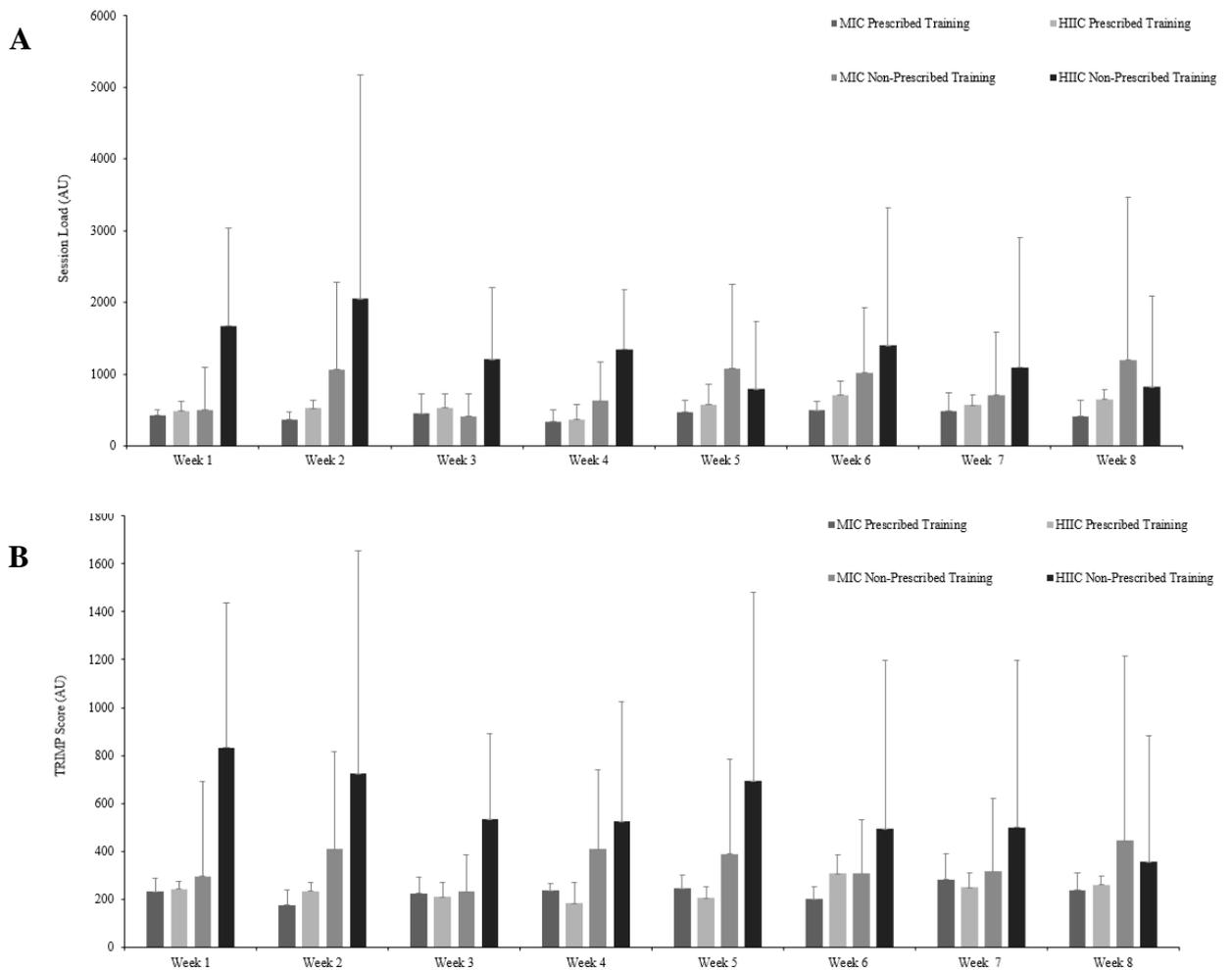
### 7.4.1 Training compliance

Training compliance was high in both groups, with  $98.2 \pm 3.0\%$  and  $98.9 \pm 2.6\%$  of total sessions completed throughout the training intervention period for the RES + HIIC and RES + MIC groups, respectively. There was no significant difference in the compliance between the two groups ( $p > 0.05$ ).

### 7.4.2 Training load

#### 7.4.2.1 Session load and training impulse score

The prescribed (laboratory) and non-prescribed (non-laboratory) session load across the 8 wk training intervention is displayed in Figure 7.3. There was no significant difference in the total prescribed session load between the RES + HIIC ( $4374 \pm 814$ ) and RES + MIC ( $3434 \pm 971$ ) conditions ( $p > 0.05$ ). This was also true of the non-prescribed session load for the RES + HIIC ( $10355 \pm 10126$ ) and RES + MIC ( $6584 \pm 4257$ ) conditions ( $p > 0.05$ ). Similarly, there was no significant difference in the total training impulse score of the prescribed or non-prescribed training amongst the two groups ( $p > 0.05$ ), as presented in Figure 7.3. Total training impulse score for the RES + HIIC group was  $1897 \pm 203$  and  $4656 \pm 4610$  for prescribed and non-prescribed activity, respectively. In comparison, the training impulse score of the RES + MIC group was  $1838 \pm 194$  and  $2804 \pm 1630$ , respectively.



**Figure 7.3.** Prescribed (laboratory) and non-prescribed (external) training load for endurance activity performed across the 8 wk training intervention, measured by the session RPE model (A) and training impulse score (B). Values presented as mean  $\pm$  SD. MIC = moderate intensity cycling group; HIIC = high intensity interval cycling group.

### 7.4.3 Body composition

#### 7.4.3.1 Body composition

There were no main effects for time or group for the change in body composition parameters from pre to post-training (Table 7.2). There were also no interaction effects across the parameters of body fat %, fat-free mass, sum of 7, sum of upper-body (UB), and sum of lower-body (LB).

**Table 7.2.** Baseline and pre to post-training change in body composition parameters for the MIC and HIIC groups.

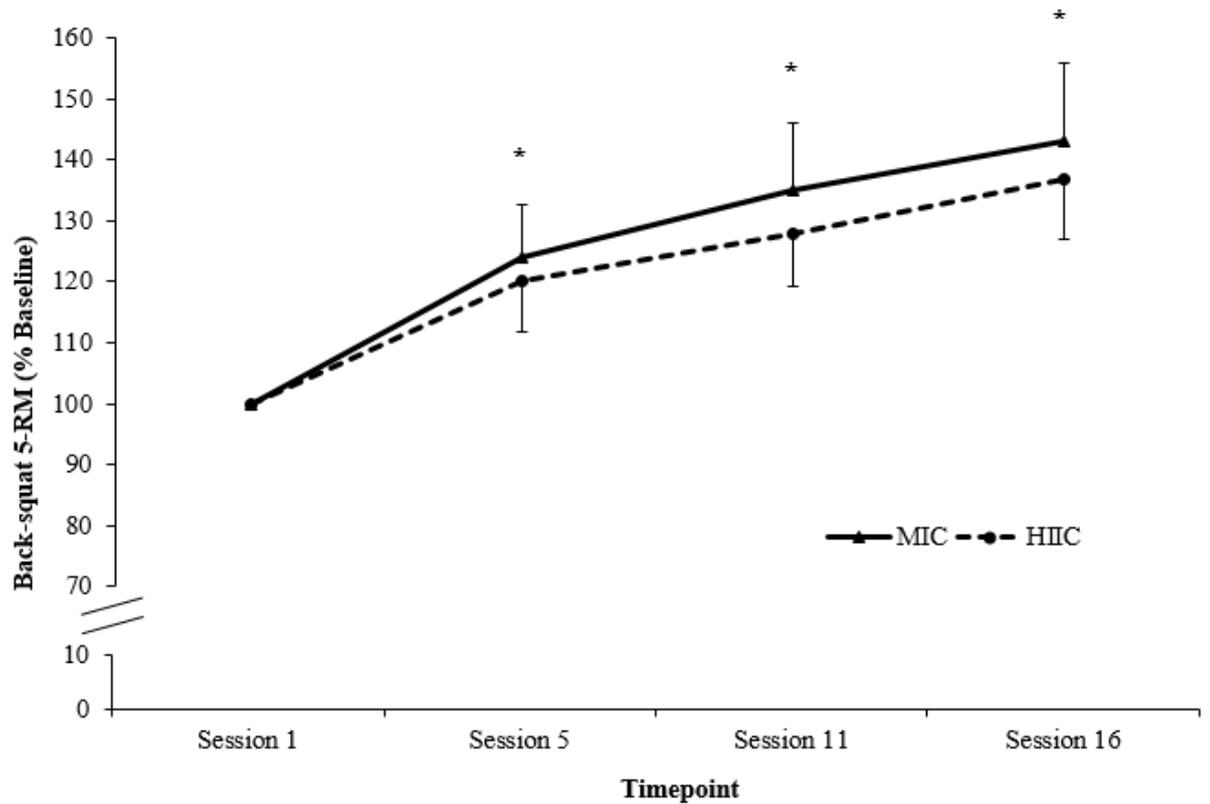
Condition	Sum of 7 (cm)	Body Fat (%)	Fat-free Mass (kg)	Sum of UB (cm)	Sum of LB (cm)
MIC (baseline)	56.6 ± 22.4	10.0 ± 3.9	54.2 ± 6.8	19.7 ± 4.9	17.6 ± 7.7
MIC (change)	1.6 ± 5.1	0.3 ± 0.9	0.8 ± 0.6	0.7 ± 1.8	-0.3 ± 1.3
HIC (baseline)	64.6 ± 14.9	11.3 ± 2.6	59.6 ± 6.8	22.0 ± 5.1	17.3 ± 4.5
HIIC (change)	2.0 ± 7.4	0.3 ± 1.3	0.2 ± 2.0	-0.2 ± 1.7	-0.4 ± 3.2

**Note:** Values presented as mean ± SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling; UB = upper body; LB = lower body

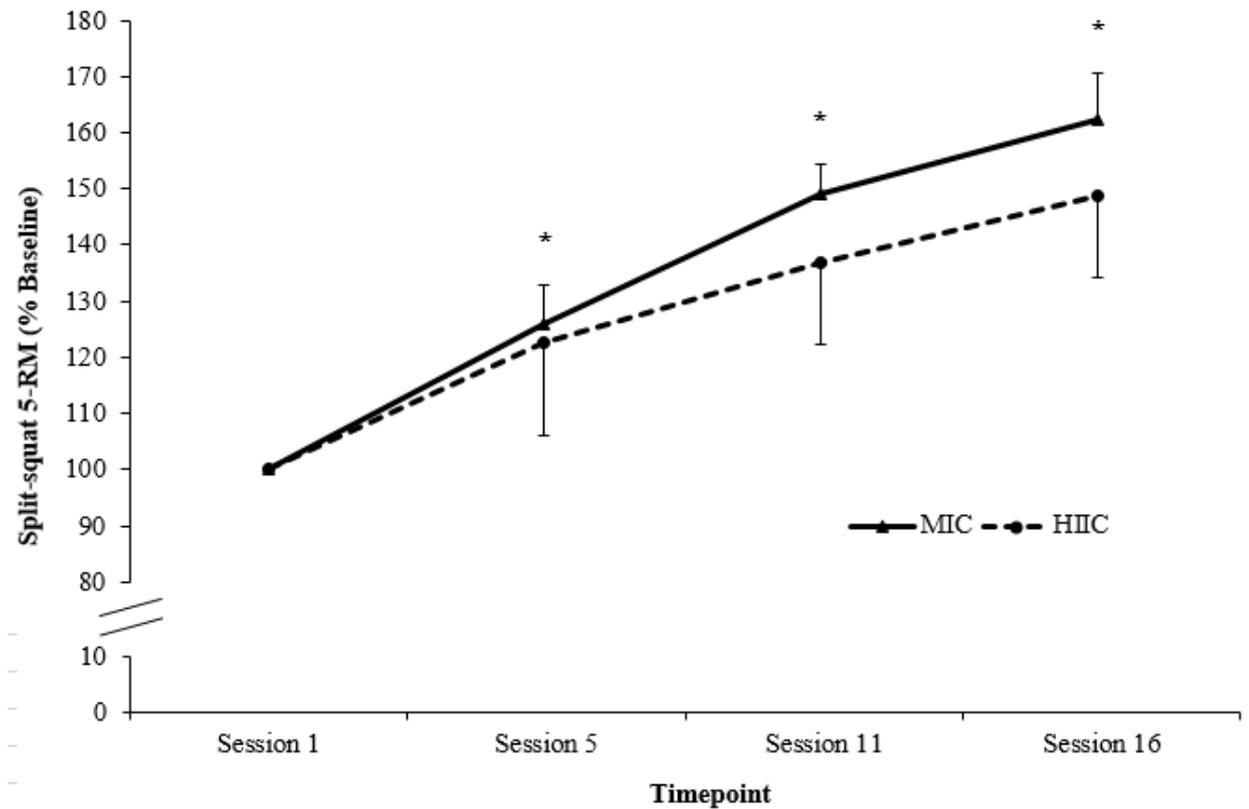
#### 7.4.4 Performance measures

##### 7.4.4.1 Maximal strength

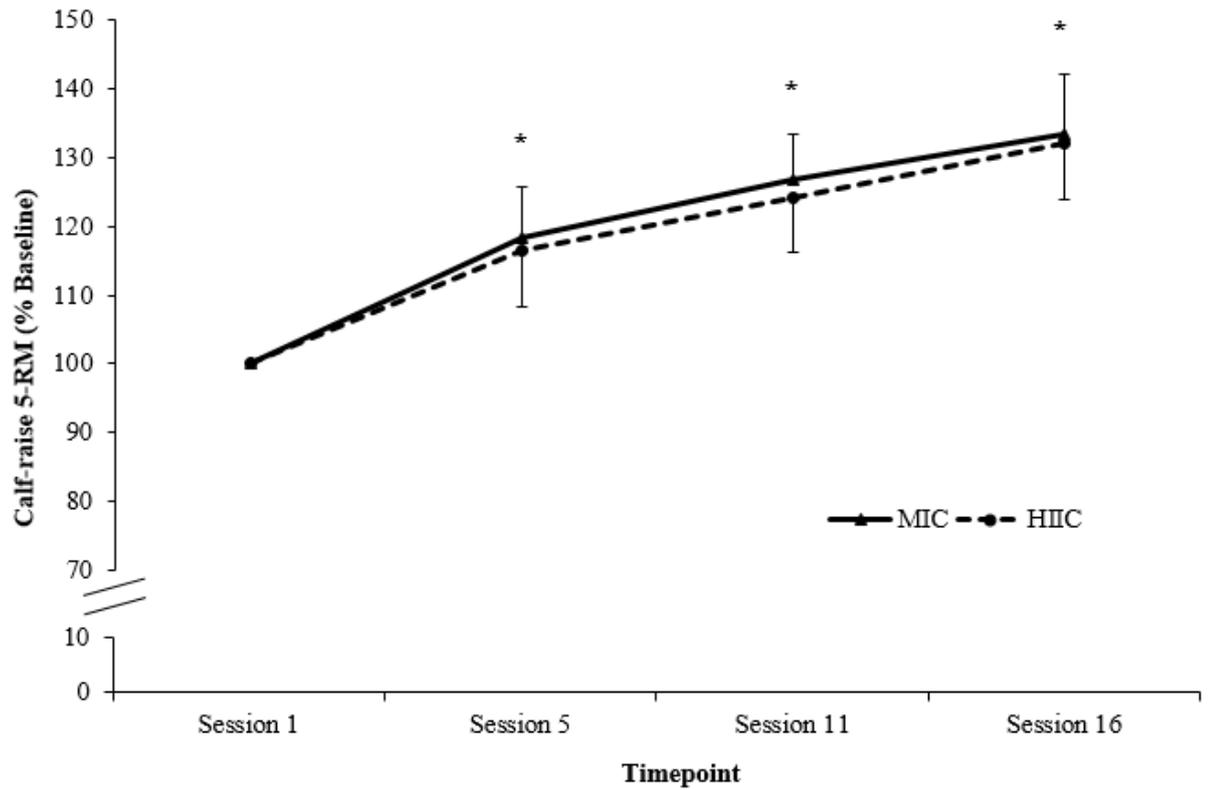
A main effect for time was observed for each of the maximal strength exercises; the back-squat ( $F_{[1,4,15,1]} = 130.590$ ,  $p < 0.001$ ), split-squat ( $F_{[2,1,23,3]} = 137.981$ ,  $p < 0.001$ ), and calf-raise ( $F_{[2,0,21,8]} = 115.410$ ,  $p < 0.001$ ). Maximal strength was greater for all time-points in comparison to the pre-training values ( $p < 0.001$ ), for all three exercises (Figures 7.4, 7.5 and 7.6). There were no group or interaction effects for any of the three exercises ( $p > 0.05$ ).



**Figure 7.4.** 5-RM back-squat performance (% change from baseline) across the intervention period in the MIC (n = 6) and HIIC (n = 7) groups. Absolute baseline values were  $89.2 \pm 14.6$  and  $95.4 \pm 22.7$  kg for MIC and HIIC, respectively. \*, significantly different from session 1 ( $p < 0.001$ ). Values presented as mean  $\pm$  SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling.



**Figure 7.5.** 5-RM split-squat performance (% change from baseline) across the intervention period in the MIC (n = 6) and HIIC (n = 7) groups. Absolute baseline values were  $51.7 \pm 9.3$  and  $56.1 \pm 7.9$  kg for MIC and HIIC, respectively. \*, significantly different from session 1 ( $p < 0.001$ ). Values presented as mean  $\pm$  SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling.



**Figure 7.6.** 5-RM calf-raise performance (% change from baseline) across the intervention period in the MIC (n = 6) and HIIC (n = 7) groups. Absolute baseline values were  $83.3 \pm 14.0$  and  $102.1 \pm 9.9$  kg for MIC and HIIC, respectively. \*, significantly different from session 1 ( $p < 0.001$ ). Values presented as mean  $\pm$  SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling.

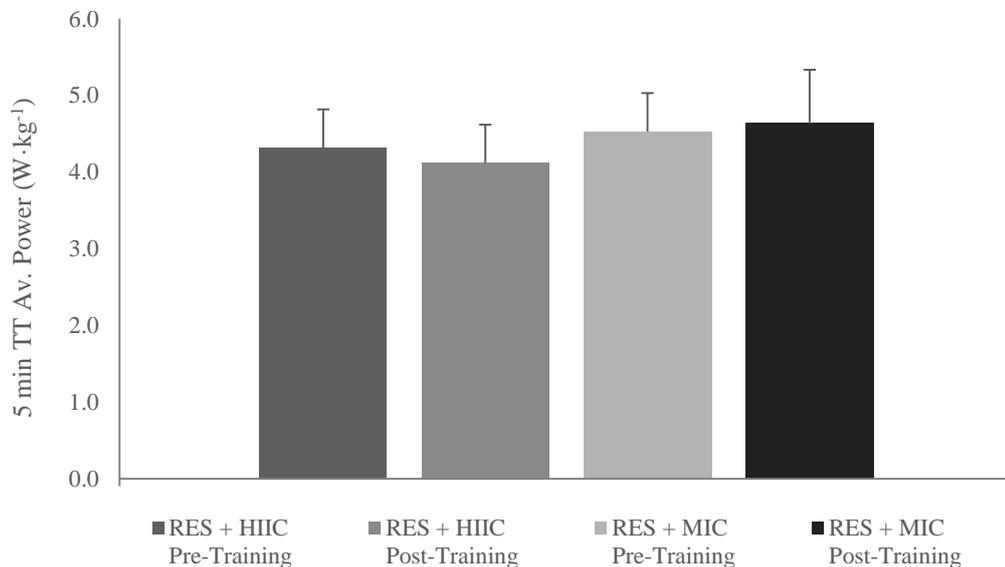
#### 7.4.4.2 Cycling specific performance

There were no significant time or group effects across the measures of power at 2 mmol·L<sup>-1</sup>, power at 4 mmol·L<sup>-1</sup>, or  $\dot{V}O_{2peak}$  (Table 7.3). Despite this, there was a time x group interaction for  $\dot{V}O_{2peak}$  from pre to post-training, with the RES + MIC group displaying a preferential response in comparison to that of the RES + HIIC group ( $F_{[1,11]} = 9.649, p = 0.010$ ). Similarly, there were no significant effects for time or group in the 5 min TT performances from before and after the training period (Figure 7.7).

**Table 7.3.** Pre to post-training change in aerobic thresholds and  $\dot{V}O_{2peak}$  for the MIC and HIIC groups.

Condition	Power at 2 mmol·L <sup>-1</sup> (W)	Power at 4 mmol·L <sup>-1</sup> (W)	$\dot{V}O_{2peak}$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )
MIC	-4.3 ± 23.3	-1.7 ± 14.1	2.2 ± 2.0
HIIC	3.3 ± 16.2	5.8 ± 18.7	-2.7 ± 3.4

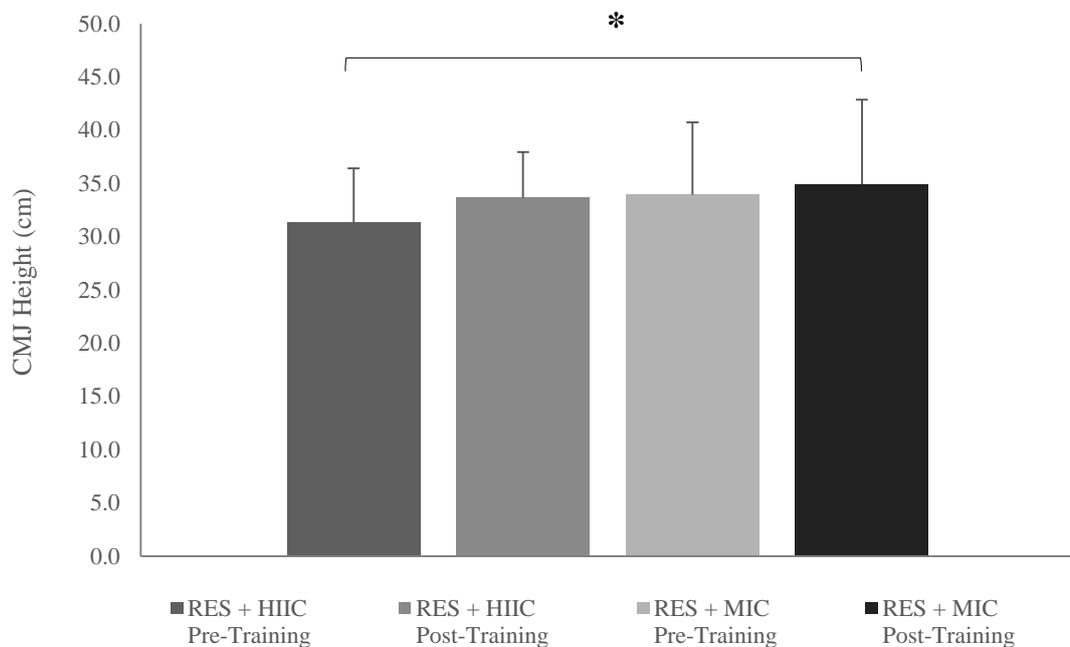
**Note:** Values presented as mean ± SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling; LT1 = lactate threshold; LT2 = lactate turnpoint



**Figure 7.7.** Mean 5 min TT performance at pre and post- intervention in the MIC (n = 6) and HIIC (n = 7) groups. Values presented as mean ± SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling.

#### 7.4.4.3 CMJ performance

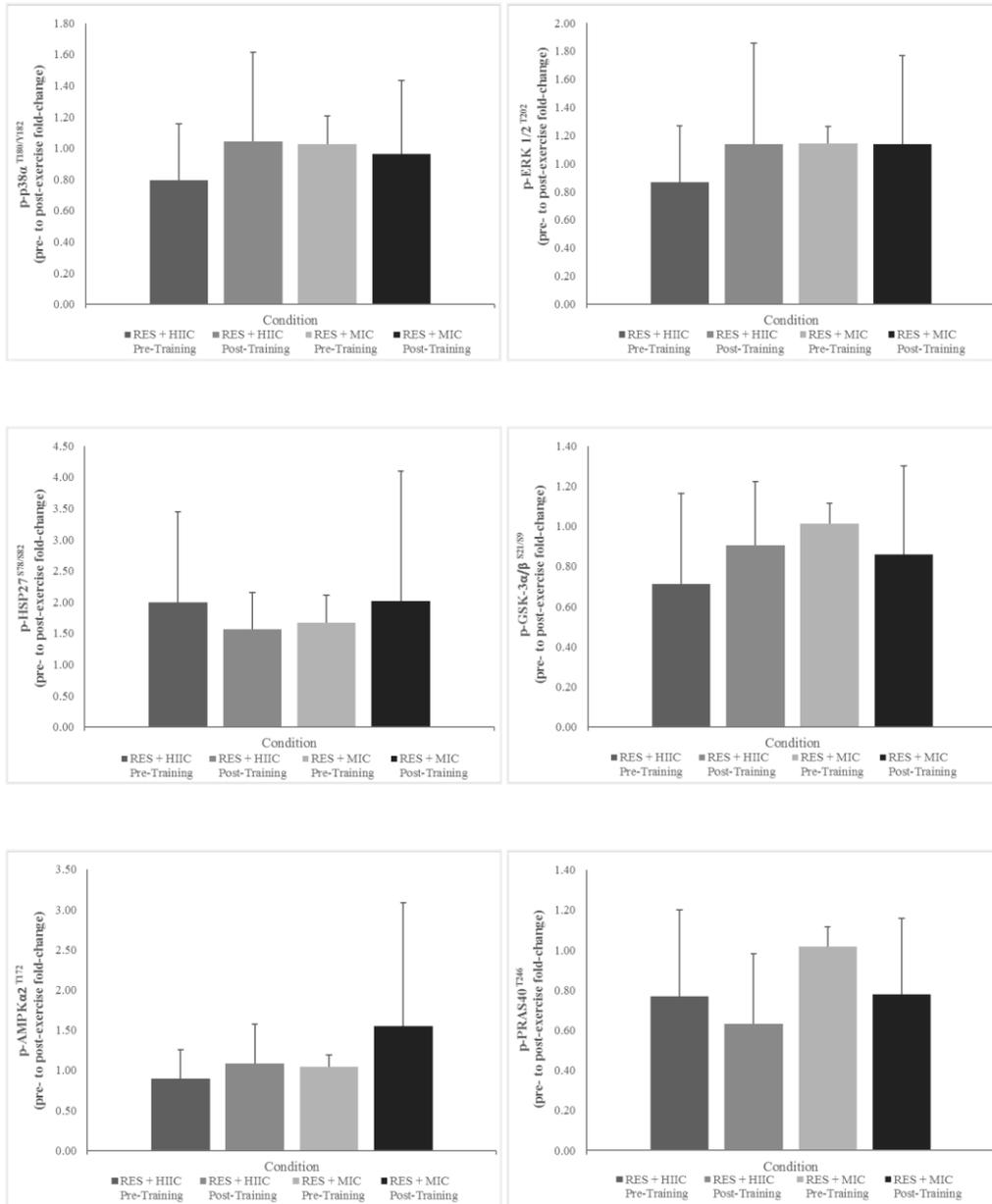
There was a main effect of time for the change in CMJ performance across the training programme ( $F_{[1,11]} = 7.849$ ,  $p = 0.017$ ), with no between group differences or interaction effects observed (Figure 7.8).



**Figure 7.8.** Mean CMJ height at pre and post- intervention in the MIC ( $n = 6$ ) and HIIC ( $n = 7$ ) groups. \*, significantly different from pre to post-intervention ( $p < 0.05$ ). Values presented as mean  $\pm$  SD. MIC = moderate intensity cycling; HIIC = high intensity interval cycling.

#### 7.4.4.4 AMPK pathway

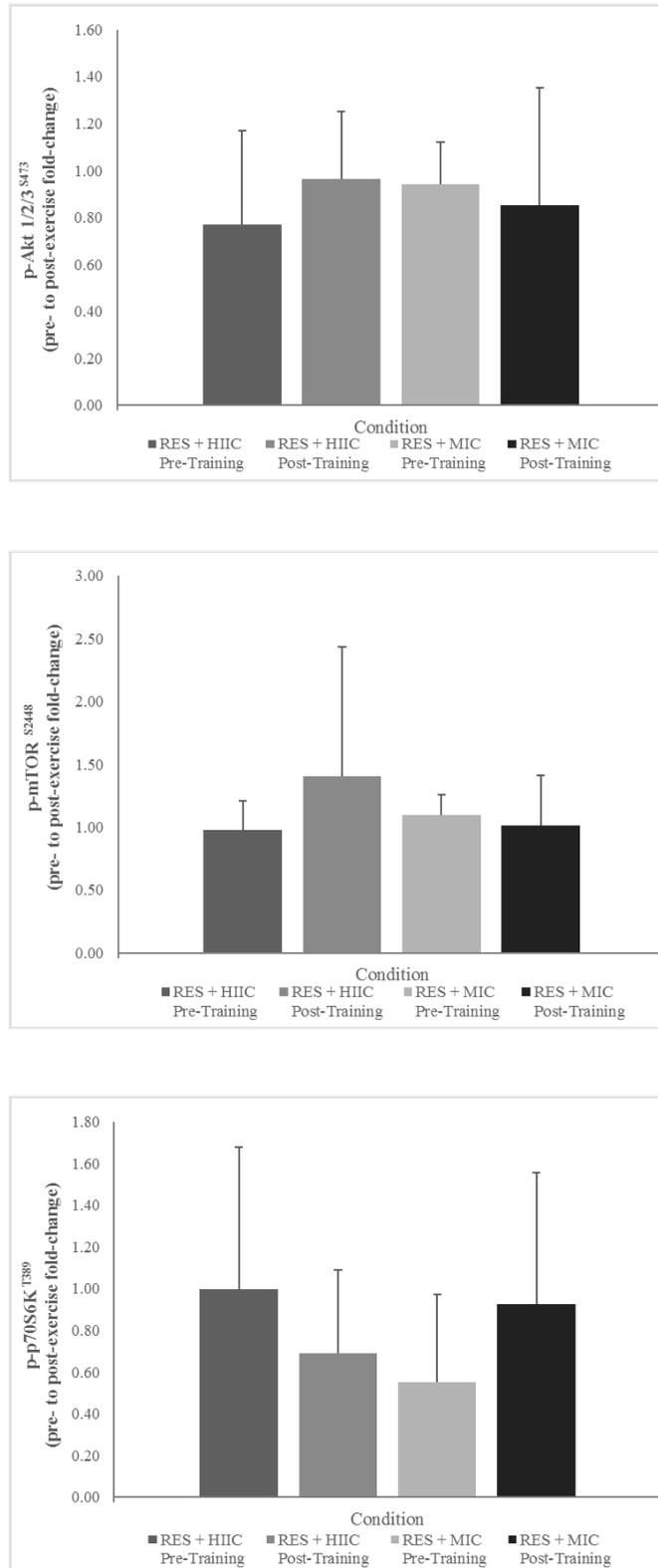
The signalling response of the protein kinases associated with the mitochondrial biogenesis pathway are presented in Figure 7.9. There were no main effects for time or group for the phosphorylation of targets in response to the single concurrent exercise stimulus conducted pre and post-training intervention ( $p > 0.05$ ). The phosphorylation of glycogen synthase kinase (GSK)-3 $\alpha/\beta^{S21/S9}$  and proline-rich Akt substrate of 40 kDa (PRAS40)<sup>T246</sup> displayed the smallest fold-change from pre to post-exercise amongst the two groups, before and after the training intervention. The greatest fold-change response was observed for the phosphorylation of HSP27<sup>S78/S82</sup>.



**Figure 7.9.** Mean response in phosphorylation of the AMPK signalling pathway in the RES + MIC (n=6) and RES + HIIC (n=7) groups. Values presented as mean  $\pm$  SD.

#### 7.4.4.5 mTOR pathway

The signalling response of the protein kinases associated with the mTOR pathway are presented in Figure 7.10. There were no main effects for time or group for the phosphorylation of targets during the single concurrent exercise stimulus conducted pre and post-training intervention ( $p > 0.05$ ).



**Figure 7.10.** Mean response in phosphorylation of the mTOR signalling pathway in the RES + MIC (n=6) and RES + HIIC (n=7) groups. Values presented as mean  $\pm$  SD.

## 7.5 Discussion

This study aimed to observe whether the intensity of endurance stimuli throughout a concurrent training block would affect performance outcomes. Further, whether the acute molecular response to concurrent exercise stimuli is affected by training status i.e. pre vs. post training, or differentially affected relative to the intensity of the endurance training prescribed. These aims were in the context of an endurance trained, but strength training naïve cohort. The major findings were that 1) the intensity of endurance stimuli had no effect on performance outcomes, despite the interventions improving strength and power parameters; 2) the acute molecular response was comparable before and after the training intervention and not differentially activated by the intensity of endurance stimuli.

Given the nature of the concurrent interference effect, the observation of strength outcomes is pertinent in research aiming to optimise concurrent training methods. Silva et al. (2012) reported no group differences in knee extension and leg press performance, with an average change across groups of 33% and 42%, respectively. These observations were specific to an untrained female cohort which had been assigned to 11 wk of concurrent training with either a continuous or high intensity endurance component. Fyfe et al. (2016a) reported slightly smaller improvements in maximal leg press strength, with a 29% and 28% change in the high and moderate-intensity conditions, respectively. These are largely consistent with the findings of this study, such that significant improvements in lower-body maximal strength were observed, with no effects relating to the endurance intensity of the concurrent stimulus. Specifically, this work observed average performance improvements of 39%, 55%, and 33% in the back-squat, split-squat, and calf-raise, respectively. The current study also used participants which were naïve to strength training, along with other similarities in methods, helping to explain the agreement in the magnitude of change. In a bid to advance the existing body of evidence and employ greater ecological validity, this work utilised applied lifting techniques to assess maximal strength. The squat and calf-raise actions are likely more familiar to strength training naïve individuals than the split-squat

exercise. There could therefore be greater potential for improvement in split-squat performance than in the other two exercise, which might be largely owing to alterations in the neural contribution to strength adaptation (Kidgell et al., 2017).

The improvements in strength were not reported in conjunction with significant improvements in fat free mass or a reduction in the sum of lower-body sites. These parameters were used as a rudimentary assessment of hypertrophy. The expectation is that 8 wk of resistance training in strength naïve individuals would likely result in improvements in strength and a surrogate assessment of hypertrophy. Therefore, these data are suggestive of neuromuscular adaptations explaining the enhanced strength performance in the respective exercises. Other research has demonstrated hypertrophy as a result of resistance training across a similar timeframe (Boone et al., 2015, Ogasawara et al., 2013). However, this observation of hypertrophy might be explained by such work incorporating more sophisticated techniques, such as magnetic resonance imaging (MRI), X-ray computerized tomography, or ultrasound. This work incorporated the skinfold technique to assess hypertrophy, but it is appreciated that this is not the ideal method to measure this parameter. Skinfold techniques have been noted to have poorer accuracy in assessing muscle CSA, in comparison to more sophisticated imaging techniques (DeFreitas et al., 2010).

While a skinfold technique has been used previously in the concurrent training literature (Silva et al., 2012), others have attempted to circumvent the associated limitations, at least in part, using DXA to quantify lean mass and infer the likelihood of muscle hypertrophy following high and moderate-intensity concurrent training interventions (Fyfe et al., 2016a). These methods displayed no change in lower-body lean mass in either of the two concurrent training groups, despite greater lean mass amongst the resistance training only intervention (Fyfe et al., 2016a). Hence, in this work and that of Fyfe et al. (2016a), hypertrophy does not appear a likely major contributing factor to improvements in strength performance following moderate and high intensity concurrent

training programmes. Instead, these changes in strength are more likely the result of predominantly neural adaptation. The nervous system plays a pivotal role in the development of strength, particularly during the initial period of strength training (Enoka, 1988, Kidgell et al., 2017). However, it is possible that there were myogenic changes subsequent to training, but that the methods employed were not appropriate to assess such alterations in the muscle. It would have been more appropriate to assess hypertrophy with an MRI technique, allowing observation of myogenic alterations.

Consistent with the response in parameters of strength, the current study observed an improvement in CMJ performance across the training intervention, with no group effects. This is in contrast to the work of Fyfe et al. (2016a), which reported no improvement for peak CMJ height amongst the two concurrent training groups. Interestingly, these authors assessed numerous aspects of CMJ performance, with the only significant improvement reported for peak velocity in the moderate-intensity group (Fyfe et al., 2016a). Power is a critical parameter in the context of concurrent training, as it is the only outcome to detrimentally change relative to resistance training in isolation, according to a previous meta-analysis (Wilson et al., 2012). Whilst it is positive that both concurrent training programmes from this study resulted in improved CMJ performance, it is not possible to place this finding in the context of the interference effect, given the design used in this work.

The two training interventions failed to improve performance across any of the endurance or cycling-specific assessments, with no benefit conveyed with either of the endurance exercise intensity programmes. This is in agreement with the vast majority of parameters assessed in the work of Fyfe et al. (2016a), other than peak aerobic power, which responded preferentially following the higher-intensity endurance stimulus. It is plausible that the additional weekly training session in comparison with this work could explain the difference in endurance performance outcomes. Others have reported a reduction in endurance performance following an investigation into the manipulation of

endurance intensity with concurrent training (Silva et al., 2012). However, these authors used a particularly poor marker of endurance performance; the maximum number of repetitions achieved at 70% 1RM. While such methods undoubtedly characterise the fatigue response of local musculature or endurance capacity, they are reported to be a poor marker of applied endurance performance (Currell and Jeukendrup, 2008). This work sought to improve ecological validity and employed a TT effort. Relative to previous research, it is unsurprising that no improvement in endurance performance was observed, given the training status of the participants. While the strength-training naïve cohort responded positively to the resistance training, it was possible that the endurance training history reduced the probability of a significant response to the endurance stimuli, owing to the law of diminishing returns. Despite this, there is evidence of similar concurrent training methods improving endurance parameters in more highly trained cyclists (Rønnestad et al., 2010, Rønnestad et al., 2017). In this study, data collection ran from the end of the racing season and is another important factor for consideration. It could be argued that helping the athletes maintain peak race form from the onset of the training intervention is a positive outcome.

While this study did not attempt to examine the role of exercise intensity in the context of an interference effect i.e. a concurrent stimulus vs. resistance only, it did examine whether endurance exercise intensity can be modified to improve a concurrent stimulus. It was important to address this question across the course of a short-term training programme. The seminal work in the field and first to examine the challenges of concurrent programming, was conducted across a short-term training intervention (Hickson, 1980). Given that the divergence in response between groups occurred from the 5-10 wk, it would seem appropriate to address such questions over at least a similar timeframe. Indeed, this consideration has been raised previously (Hamilton and Philp, 2013), with authors stressing the requirement for research observing the molecular responses across a longer timeframe than the popular model of acute observations.

Previous efforts to observe molecular responses to concurrent stimuli across a period of greater than 5 wk are limited. de Souza et al. (2013) reported total p70S6K and phosphorylated Akt protein expression to increase from pre to post-training time points. Similarly, Fyfe et al. (2018) observed greater basal phosphorylation of p70S6K, with both mTOR and rpS6 phosphorylation still increasing in response to concurrent exercise following 8 wk of training. Conversely, Kazior et al. (2016) reported a reduction in total p70S6K content, but in combination with an increase in mTOR and Akt protein expression post-intervention. The literature has characterised the responses amongst recreationally active individuals, with none of the methods specifically comparing the acute response to concurrent exercise before and after a training intervention. Arguably, a design of this nature would better support conclusions regarding the role of training status in the molecular response to acute concurrent exercise. The importance of training status and its ability to modulate both the specificity and magnitude of training adaptations has previously been described in the literature (Fyfe and Loenneke, 2018).

Fernandez-Gonzalo et al. (2013) utilised an, arguably, improved design and assessed acute molecular responses to a concurrent stimulus in both the pre and post-training condition. While the activation status of mTOR, rpS6 and eEF2 remained unaltered, p70S6K phosphorylation increased in the trained state (Fernandez-Gonzalo et al., 2013). This would counter the hypothesis of an attenuation in, or a more mode-specific response to, exercise in the trained state. However, these findings were in the context of 5 wk of training amongst moderately active students, and therefore not reflective of a prolonged training history. The major finding from this study was a lack of a time effect in protein phosphorylation fold-change from pre to post-intervention. This consistency in early exercise response before and after the training intervention is suggestive of either 1) continued adaptation after 8 wk of training, or 2) a poor exercise stimulus from the onset of the intervention. The former seems more likely in this scenario given the improvement in strength and power parameters.

Previous literature concerning the role of endurance exercise intensity during concurrent training has employed an endurance followed by resistance exercise order for the concurrent training stimulus (Fyfe et al., 2016a, Silva et al., 2012). Employing this exercise sequence might stress the neuromuscular element of residual fatigue within a concurrent training paradigm (Lepers et al., 2000). However, the meta-analysis from Chapter 5 indicates a beneficial effect of a resistance followed by endurance exercise order for lower-body strength adaptation across a short-term concurrent training programme. This would suggest that such an exercise sequence provides an appropriate model to examine the optimisation of concurrent training methods. It is the development of strength which is potentially inhibited with this training paradigm, and as such, the methods should strive to elicit adaptation in strength parameters. This would constitute a more ecologically valid paradigm to investigate the role of endurance exercise intensity and is the model adopted in this work.

This work does not support the idea of endurance exercise intensity negatively modulating the adaptive response of resistance exercise structured in a short-term concurrent training paradigm. This is in agreement with previous work in untrained cohorts (Fyfe et al., 2016a, Silva et al., 2012) and could support the concept of volume or frequency of endurance stimuli proving a more potent mediator of adaptation to concurrent training (Jones et al., 2013). While the design of the work does not confirm whether either endurance training condition had an inhibitory effect on strength adaptation, the magnitude of strength adaptation observed is similar compared with that reported following short-term resistance training in strength naïve individuals (Campos et al., 2002, Del Balso and Cafarelli, 2007). Further, the complexities of research design in concurrent training literature should also be considered. There are many acute training variables encountered when implementing a concurrent training paradigm; such as intensity, volume, sequence, exercise relief, and frequency. While this study manipulated the variable of intensity and attempted to control for other components, it is not possible to identify the effect of

employing alternate conditions with regards to these variables, and the resultant outcome on performance. It should also be acknowledged that while the group difference in non-prescribed endurance training load did not reach statistical significance, it could be physiologically relevant. Furthermore, while ensuring control by delivering comparable work-matched endurance stimuli, the ecological validity of work-matched endurance interventions in trained cohorts has been questioned (Seiler and Tønnessen, 2009a). This work provides valuable information regarding the response to HIIT at 85%  $\dot{V}O_{2\text{peak}}$ , which represents a training stimulus that athletes might undertake, however caution should be exercised in extrapolating these findings to interval training of higher intensities, such as  $\dot{V}O_{2\text{max}}$ .

## **7.6 Summary**

This study examined whether the intensity of endurance stimuli throughout a concurrent training block would affect performance outcomes. Further, this training study observed whether the acute molecular response to concurrent exercise stimuli is affected by training status i.e. pre vs. post, or differentially affected in relation to the endurance intensity prescribed throughout the training intervention. It was confirmed that the intensity of endurance exercise (as part of a concurrent training stimulus) had no effect on performance outcomes, following short-term concurrent training. Importantly, this was in the context of improvements in strength and power parameters. Further, the acute molecular response to a concurrent exercise stimulus was comparable before and after the training intervention, suggesting that training status had no effect on the molecular responses assessed. Finally, the molecular responses to a concurrent exercise stimulus were not differentially activated by the intensity of endurance stimuli. This work addresses a specific aim of the thesis, by assessing whether endurance exercise intensity modifies the adaptation in lower-body strength and cycling-specific endurance following concurrent training, and

whether the acute response to a concurrent stimulus is modified in a concurrent-trained state.

## **8. GENERAL DISCUSSION**

## **8.1 Aims and objectives of this thesis**

The overall aim of this thesis was to examine and elucidate the physiological and molecular responses to concurrent training; a training paradigm associated with an interference effect (Hickson, 1980). The sequential studies were designed with the objectives of profiling the recovery process following a strenuous concurrent exercise stimulus, before clarifying the role of two prominent training variables; intra-session exercise sequence and endurance exercise intensity, with regards to how these variables might act to present an interference effect, or be modified to provide superior adaptive responses. As such, the intended application was for the data to prove beneficial for those individuals (athletes and practitioners) aiming to better understand the responses to, and potential for, concurrent training. More specifically, how this training paradigm might be optimised for the endurance-trained athlete.

## **8.2 Chapter reviews**

### *8.2.1 Chapter 4*

This investigation examined the response to a strenuous bout of concurrent exercise. Specifically, the profile of recovery within an EIMD context and whether nutritional supplementation strategies would act to better support this process. Moreover, both mechanistic and applied parameters were employed to ascertain the effect of performing a challenging concurrent stimulus, amongst endurance-trained cyclists. Given the population, an endurance stimulus with high ecological validity was employed, along with a resistance stimulus of low technical demand. Finally, great rigour was employed regarding the content of the nutritional supplementation and the habitual diet throughout the experimental period.

The primary finding was muted performance decrements in comparison to those from single mode EIMD literature. It was postulated that this finding might have been influenced by the metabolic challenge of the cycling protocol, performed prior to the resistance exercise stimulus. Further, these data failed to support the efficacy of protein supplementation to benefit the recovery process. This could have been as a result of the provision of a quality habitual diet and/or appropriate supplemental controls, or indeed, the relatively modest indices of EIMD imposed by the concurrent exercise.

### *8.2.2 Chapter 5*

The findings of Chapter 4 highlighted the potential for the sequence of concurrent exercise to modify the response to training stimuli. Therefore, the purpose of this study was to examine, with a systematic review and meta-analysis, the role of exercise sequence within the context of the concurrent training interference effect. More specifically, the aim was to determine whether intra-session exercise sequence affected the outcomes of lower-body dynamic and static strength, lower-body power and muscle hypertrophy, maximal aerobic capacity, and body fat %. Given the potential for exercise sequence to influence an interference effect and the equivocal nature of the body of evidence, it was deemed important to perform a robust systematic review and meta-analysis, to provide greater clarity.

It was confirmed that intra-session exercise sequence is an important training variable within the context of the interference effect, with a resistance-endurance exercise order proving beneficial for improvements in lower-body dynamic strength. There was no support for a given exercise order across a concurrent training programme for the other outcomes assessed. Despite this, the finding for lower-body dynamic strength is pertinent, owing to its more direct application to the performance environment in comparison to some of the other outcomes assessed. Given that an order effect was only observed for one outcome, it was recommended that individuals limited by time, such that they must train

concurrently with minimal relief between modes of exercise, follow a resistance-endurance exercise order.

### 8.2.3 Chapter 6

The findings of Chapter 5 emphasised that manipulating acute training variables might help to optimise adaptation to concurrent training. The direction of the thesis from this point, was to focus on another training variable; endurance exercise intensity, in both an acute and chronic scenario. Hence, the purpose of this study was to observe whether the intensity of the endurance stimulus might affect the activation status of signalling proteins associated with the mTOR and AMPK networks. Further, the study was designed to examine whether a molecular interference was observed amongst trained endurance cyclists, which were strength training naïve. All concurrent stimuli from this point forwards followed a resistance-endurance exercise order, to ensure a stimulus appropriate for maximising the strength response, based on the findings from Chapter 5.

The primary finding was a lack of support for an acute molecular interference effect in a trained endurance cohort. Further, in an acute context, the data failed to support the intensity-dependent regulation of AMPK, nor differential activation of the anabolic machinery with the manipulation of endurance exercise intensity. These data suggested that endurance athletes might not need be concerned with the intensity of their endurance session (moderate *vs.* high intensity) affecting their strength adaptation, when the two exercise modes are performed in close proximity to one another. Caution should be exercised however, as these findings are specific to an acute context. This question should be examined across a training period, in order to provide greater confidence in the longer-term effects. Particularly so, given that phosphorylation of the mTORC1 and AMPK networks could prove poor correlates for chronic outcomes (Miller et al., 2016), likely owing to the complex interactions involved with concurrent training practice.

### 8.2.4 Chapter 7

Given the findings of Chapter 6, it was considered important to examine whether the intensity of endurance stimuli throughout a concurrent training block would affect performance outcomes. This would enable greater confidence in the practical application of manipulating this training variable, for the population in question. Further, the previous chapter highlighted the uncertainty in using molecular data as a proxy for training outcomes. This training study afforded the opportunity to observe whether the acute molecular response to a concurrent exercise stimulus is affected by training status i.e. pre vs. post, or differentially affected in relation to the endurance intensity prescribed throughout the training intervention. Importantly, the latter could be viewed against the chronic training outcomes.

It was confirmed that the intensity of endurance exercise (as part of a concurrent training session) had no effect on performance outcomes, following short-term concurrent training. Importantly, this was in the context of improvements in strength and power parameters. Further, the acute molecular response to a concurrent exercise stimulus was comparable before and after the training intervention, suggesting that training status had no effect on the molecular responses assessed (acknowledging the limited timeframe of the intervention). Finally, the molecular responses to a concurrent exercise stimulus were not differentially activated by the intensity of an endurance stimulus. These findings were in agreement with the acute observations from Chapter 6, in that moderate and high intensity endurance stimuli provide a similar strength stimulus during concurrent training. Importantly, this study confirmed that strength and power adaptations were possible with either endurance stimulus. Surprisingly, these improvements in strength and power were not elicited in conjunction with improvements in cycling-specific performance.

## **8.3 General discussion**

### *8.3.1 Role of programme variables*

The majority of the research conducted as part of this thesis focussed on the manipulation of acute programme variables, to better understand the impact on the interference effect or parameters associated with this phenomenon. Specifically, the training variables of exercise sequence and intensity were selected for observation. Despite a growing literature base concerning the effect of exercise sequence on strength adaptation within a concurrent training paradigm, much conjecture remained. Further, despite extensive research concerning the concurrent training paradigm and the interference effect, few studies have investigated the potential for endurance exercise intensity to determine the presence of an interference effect or modulate strength and power training outcomes.

#### *8.3.1.1 Sequencing of exercise*

The findings from Chapter 4 highlighted the potential for intra-session exercise sequence to influence the acute response and recovery profile from a concurrent exercise stimulus. This prompted the subsequent research question addressed in Chapter 5; whether the sequence of exercise in a concurrent training paradigm determined the magnitude of adaptation in a chronic context. The primary finding from Chapter 5 indicates that performing a resistance-endurance exercise order enhanced the improvement in lower-body dynamic strength within a prolonged concurrent-type training programme. In contrast, lower-body static strength, muscle hypertrophy, maximal aerobic capacity, and body fat % were unaffected by intra-session exercise sequence.

It is possible that the superior response following a resistance-endurance exercise sequence is explained by the residual fatigue theory, presented as part of the acute hypothesis. In short, the fatigue or muscle milieu following the endurance stimulus leads to a sub-optimal strength stimulus and attenuates adaptation over time. This process might be

explained by either an attenuated activation status of anabolic signalling targets in a glycogen depleted state (Creer et al., 2005), or indeed, an acute reduction in strength output following endurance activity (Jacobs et al., 1981, Lepers et al., 2000). It is curious that there is little mention in the concurrent literature, for the potential of strength activity to present residual fatigue for subsequent endurance performance (Doma and Bede Deakin, 2013), particularly if the strength stimulus precipitates EIMD (Burt and Twist, 2011b). This could be explained by the nature of the interference effect; i.e. strength attenuation with the practice of both exercise modes. As such, the focus for research has been to better understand the mechanisms which limit strength performance. However, this question remains to be fully elucidated and could provide insight into optimising concurrent training methods, particularly for endurance athletes. If residual fatigue is presented by resistance-endurance exercise sequence, the results from Chapter 5 would suggest that it is less than that presented with an endurance-resistance exercise order, if the acute hypothesis holds true.

In the context of the chronic hypothesis, it was suggested that neuromuscular mechanisms might explain the preferential response in strength adaptation with a resistance-endurance exercise sequence. Cadore et al. (2013) postulated that greater adaptation in lower-body dynamic 1-RM with a resistance-endurance exercise sequence might be attributed to improved neuromuscular economy, owing to improvements in strength and reduced EMG signal for a given load. A suggested role for adjustments in neuromuscular function is also supported by Eklund et al. (2015), with increased maximal force in combination with an increase in muscle activation in the resistance-endurance training group only.

There are insufficient data to suggest a role for molecular responses to explain the finding for lower-body dynamic strength to be altered by exercise sequence. The same research group observed the greatest Akt phosphorylation with an endurance-resistance order (Coffey et al., 2009b), but order effects for p70S6K phosphorylation, with

preferential activation when the strength stimulus preceded sprint intervals (Coffey et al., 2009a). While these disparate findings might be explained by the difference in the cycling stimulus i.e. continuous vs. 10 x 6 s sprint intervals, these data do not provide evidence for acute responses in the mTOR pathway to explain the role for exercise sequence to modify strength. Further, more recent data suggested a similar post-exercise signalling response, irrespective of exercise order (Jones et al., 2015).

The meta-analysis performed in Chapter 5 was the first to address the question of whether exercise sequence can mitigate/exacerbate a potential interference effect. It is important to stress that the findings from Chapter 5 do not solely offer a role in causal inference, but also provide an assessment of the consistency of results reported at an individual study level and greater precision of the summary effect outcomes. As such, the findings report a pooled mean difference of 6.9% for lower-body dynamic strength, in favour of a resistance-endurance exercise order. While this was the only outcome moderated by exercise sequence, it is pertinent to clarify that power could not be included in the meta-analysis, owing to insufficient data. This training outcome has previously been reported to be most sensitive to the interference effect (Wilson et al., 2012) and the potential for it to be modulated by exercise sequence remains an important research question.

### *8.3.1.2 Intensity of exercise*

The primary finding from Chapter 6 was that AMPK phosphorylation was not regulated in an intensity-dependent manner, corresponding with the mTOR pathway response, which was also unaffected by the intensity of the endurance stimulus during concurrent exercise. While this addressed a potential role for the intensity of endurance exercise to modulate the acute molecular response amongst endurance-trained athletes, it was deemed important to explore this question in relation to longer-term adaptive responses, across a training intervention period; subsequently addressed in Chapter 7. The

major findings were that the intensity of endurance stimuli had no effect on performance outcomes, despite both concurrent training programmes improving strength and power parameters, and further, that the acute molecular response was comparable before and after the training intervention and not differentially activated by the intensity of endurance stimuli.

The findings from chapters 6 and 7 indicate that the programme variable of endurance exercise intensity does not regulate the short or longer-term responses to concurrent training. This finding is largely consistent with previous research (Fyfe et al., 2016a, Fyfe et al., 2016b), albeit in a different population, with this thesis providing data specific to an endurance-trained cohort. This was important, given that training status has been suggested to effect the molecular response to exercise stress, with an attenuated response in a training-accustomed phenotype (Coffey et al., 2006). However, HIIT is reportedly able to increase the phosphorylation of AMPK in response to exercise, even amongst highly-trained endurance athletes (Clark et al., 2004). Despite this, the data failed to suggest greater phosphorylation of AMPK with HIIT, nor the observation of a molecular interference amongst an endurance-trained cohort.

While acute molecular responses can provide insight into the potential for longer-term adaptive responses, it was deemed important to address the role of exercise intensity across a training intervention period. The findings from Chapter 7 indicate that it is possible to improve strength and power parameters with both moderate and high intensity endurance stimuli, as part of the concurrent exercise stimulus. This is an agreement with previous literature, with no differential effect on performance outcomes according to endurance exercise intensity (Fyfe et al., 2016a, Silva et al., 2012). While addressing several of the previous limitations, the data from Chapter 7 still suffers from a lack of control for non-prescribed endurance training load. This poses a threat to construct validity and future work should strive to eradicate this. Previous research has reported both a decline (Silva et al., 2012) and improvements (Fyfe et al., 2016a) in parameters of endurance performance,

irrespective of endurance exercise intensity. The work from this thesis observed no time effects for any of the parameters of endurance performance, irrespective of training group. While this is naturally important for the endurance athlete, others have reported no improvement in cycling TT performance with the addition of strength training, specifically in elite endurance athletes (Rønnestad et al., 2017). It is possible that the lack of improvement in endurance performance observed in this thesis, could be explained by differences in training status compared to previous work, or indeed, that the onset of the training intervention coincided with the height of the competitive season i.e. no room for improvement. The lack of time effects could have represented maintenance of peak form with the concurrent training programme.

### *8.3.2 Mechanisms relating to the interference phenomenon*

Beyond the aims of better understanding the role of distinct training variables, this thesis sought to investigate a potential mechanism associated with the interference phenomenon, and therefore implicated in modifying the strength and power outcomes with concurrent training practice. In particular, the thesis included methods allowing the observation of molecular signalling responses following concurrent exercise, with a particular focus on how exercise intensity and training status might impact these responses.

#### *8.3.2.1 Molecular factors*

The data from Chapter 6 indicate that despite the observation of differential AMPK and mTOR signalling between conditions, this was not suggestive of an interference effect or the intensity-dependent regulation of AMPK. Specifically, the elevated phosphorylation of AMPK in the RES + MIC vs. RES conditions did not coincide with an inhibition of mTOR, but rather, a corresponding increase in the phosphorylation of mTOR. This contrasts with the inhibitory cross-talk previously observed in murine models (Atherton et al., 2005), as does a great deal of previous research in humans; with data to refute elevated AMPK acting to inhibit mTOR phosphorylation (Apro et al., 2015) and data indicating

greater anabolic responses following concurrent *vs.* resistance stimuli (Lundberg et al., 2012, Pugh et al., 2015). Beyond data displaying inconsistencies with the premise of the ‘AMPK-PKB(Akt) switch’ and the interference effect, other investigations have reported no difference in the activation status of mTOR and AMPK signalling networks following concurrent exercise stimuli (Apro et al., 2013, Jones et al., 2015).

The data from chapters 6 and 7 revealed modest responses in the phosphorylation of specific effectors of the AMPK and mTOR signalling networks in comparison to some (Apro et al., 2015, Apro et al., 2013, Wang et al., 2011), but not all of the previous research in the field (Apro et al., 2013, Lundberg et al., 2012, Pugh et al., 2015). Where inconsistencies are present in comparison to previous literature, these are likely explained by methodological differences from those used in this thesis. Concurrent training employing a running *vs.* cycling endurance stimulus is reported to produce a smaller effect size for strength and hypertrophy, with interference in these parameters only observed between running concurrent and strength only conditions (Wilson et al., 2012). The intra-session relief period is also of importance to training outcomes, with a 6 h relief period beneficial for strength adaptation in comparison to within session concurrent stimuli *i.e.* no relief period (Robineau et al., 2016). A within session concurrent model was employed in all of the experimental chapters of this thesis. Further, the training status of participants is suggested to influence both the magnitude and specificity of the acute and chronic skeletal muscle responses to a concurrent stimulus (Fyfe and Loenneke, 2018). The numerous methodological permutations afforded with a concurrent training paradigm clearly presents a challenge in the comparison of data across research studies, with inconsistencies in results explicated by multiple variables.

While the observation of acute molecular signalling responses provides a fascinating insight into potential responses in training adaptation, the appropriateness of protein phosphorylation to act as a proxy for longer-term adaptive processes should still be treated with an element of caution. Indeed, prominent authors in the field have commented

on the underwhelming knowledge gleaned from using isolated effectors to investigate the interference phenomenon, instead advocating a more integrated approach (Coffey and Hawley, 2016). Others have criticised the approach of focusing on an individual element of the transduction – transcription - translation process, to infer the result of the exercise adaptation (Miller et al., 2016). This is primarily owing to the way in which individual steps of the process are independently regulated and the potential for subsequent modification. Approaches with multiple outcomes aligned to post-transcriptional assessments, fraction-specific measurements of muscle protein synthesis, and indeed exercise adaptations observed through training studies, would help to provide greater insight into the effects of exercise on skeletal muscle. This approach of employing the assessment of molecular targets as supplementary data to the adaptive response of skeletal muscle, was used in Chapter 7 of this thesis.

#### *8.3.2.2 Training status*

Training status is suggested to influence both the magnitude and specificity of the acute and chronic skeletal muscle responses to concurrent stimuli (Fyfe et al., 2018). Chapters 6 and 7 provide new information concerning the response of an endurance-trained population to concurrent exercise stimuli. In particular, the research design in Chapter 7 enabled the observation of the acute molecular response before and after an 8 wk training intervention. The acute molecular response was comparable before and after the training programme, indicating a similar stimulus at the two time points. The strength training history of the cohort was the primary parameter modified during this period i.e. endurance-trained, but strength-naïve at the onset of the intervention. The improvement in strength and power performance across the training study would suggest that the stimulus at 8 wk was still effective, given that the acute response was comparable to pre-training and coincided with improvements in performance. It is suggested that the naivety to strength stimuli is to explain for these performance outcomes improving exclusively, with the endurance training history of the cohort reducing the potential for adaptation across these parameters.

The observation of comparable early exercise signalling responses before and after the training intervention was unexpected. Training status has been observed to modify the early molecular signalling responses to opposing exercise stimuli (Coffey et al., 2006). Specifically, an attenuated molecular response is proposed amongst trained phenotypes, with a generic molecular footprint response in untrained cohorts (Coffey and Hawley, 2016). Hence, it was thought that the response in the anabolic machinery would be more likely to display time effects, with an attenuation following the training intervention. Despite this, others have reported increased activity in effectors of the mTOR pathway following a training intervention, albeit just a 5 wk concurrent training programme (Fernandez-Gonzalo et al., 2013). These data counter the hypothesis of an attenuated molecular response to exercise with an improved training status.

## **8.4 Practical applications**

Observations from this thesis should prove useful to athletes and practitioners alike, whom are interested in better understanding the response to, and performance decline following, intense concurrent stimuli. Further, the findings should provide insight as to whether training variable manipulation bears an adaptive cost, when attempting to improve outcomes previously associated with the interference phenomenon. The specific training variables of interest were exercise sequence and endurance exercise intensity. The findings should prove of particular relevance to endurance-trained athletes, whom have a clear rationale for conducting concurrent training.

Completing an intense bout of concurrent training resulted in performance decrements for up to 48 h, particularly if the strength element of the stimulus necessitates eccentric muscle actions. Furthermore, it is unlikely that the recovery process is accelerated with protein supplementation, provided that a quality habitual diet is consumed. This likely indicated that when athletes must train concurrently with minimal relief between exercise

modes, care must be employed regarding the proximity of subsequent sessions where high quality/intensity is required. Although not specifically assessed in this thesis, previous research suggested preceding endurance activity will act to limit the quality of a strength stimulus (Lepers et al., 2000), and perhaps limit muscle damage and attenuate performance decrements. It is possible that the practitioner could use this knowledge strategically within a training block.

Given the existence of inevitable logistical constraints, it is highly likely that athletes and recreational exercisers alike will complete their strength and endurance training in close proximity to one another. As such, it is important to understand whether the order of these exercise modes has implications on the possible physiological adaptations. The findings of this thesis support the practice of a resistance followed by endurance exercise order for the training outcome of lower-body dynamic strength, during a prolonged ( $\geq 5$  wk) concurrent training programme. Other outcomes such as static strength, muscle hypertrophy, maximal aerobic capacity, and body fat % were not affected by exercise sequence. In the majority of athletic scenarios, dynamic strength expression is more prevalent than that of isometric strength, and therefore likely to be more meaningful to the athlete and practitioner. Given that an order effect was only observed for one outcome, it is recommended that individuals limited by time, such that they must train concurrently with minimal relief between modes of exercise, follow a resistance-endurance exercise order. Given the cohorts included in the meta-analysis of Chapter 5, the conclusions are particularly relevant to recreational exercisers or untrained individuals.

The role of endurance exercise intensity within the concurrent training paradigm does not seem of paramount importance for subsequent adaptation. When choosing between moderate steady-state and high intensity interval endurance stimuli, there appears to be no effect on the early molecular responses or the longer-term adaptive responses associated with strength and endurance performance. Importantly, this relates specifically to work and duration-matched conditions and examples of higher or lower intensity

endurance stimuli could produce different outcomes. The findings indicate the potential of a relatively short concurrent training block, irrespective of endurance exercise intensity, to improve strength and power parameters amongst trained endurance athletes. This was in contrast to endurance performance outcomes, which remained stable. It is likely that these observations relate to the training status of the athletes; i.e. endurance-trained but strength naïve, and the law of diminishing returns, possibly in combination with the timing in the competitive season. While both the molecular and training outcome data were similarly unaffected by the prescription of exercise intensity, the molecular data did not reflect the improvements in strength and power performance. Taken together, these findings suggest that the observation of molecular signalling responses in isolation are not sufficient to represent the longer-term training outcomes following concurrent training.

## **8.5 Limitations**

It is acknowledged that work from this thesis does possess limitations. It was intended that findings from this thesis provide strong application for athletes and practitioners alike. Despite this, the resistance exercise stimulus used in Chapter 4 is limited in this regard, with the use of a drop-jump resistance protocol as opposed to an Olympic lifting protocol. However, while the protocol does lack some external validity, it does provide some application; providing an exercise that individuals can engage in, in comparison to an eccentric resistance stimulus using a dynamometer, for example. Further, the protocol was employed due to a lower cognitive/ skill demand, which was deemed more appropriate given the training status of the participants. While Chapter 5 examines the literature concerning the role of exercise sequence within a concurrent training paradigm, in order to provide greater precision in the summary of effect outcomes, these outcomes should still be interpreted with a level of caution. Some of the selected outcome measures possessed moderate to substantial levels of heterogeneity. The associated level of

inconsistency in the results of individual studies could be troublesome for providing clarity in recommendations. However, this highlights the complexities of a concurrent training paradigm, with the inconsistencies likely a representation of the different methods made possible with the numerous acute training variables involved. The purpose of Chapter 7 was to examine whether the intensity of endurance stimuli throughout a concurrent training block would affect performance outcomes. Given this aim, it was imperative to control numerous factors. Despite this, it was not possible to control the endurance training load that the participants completed outside the laboratory environment. While this factor has not been controlled in previous work in the literature (Fyfe et al., 2016a) and the discrepancy in load was not significant in this thesis, this still remains a limitation of the work.

## **8.6 Future research**

Whilst findings from this thesis provide information regarding the responses to and adaptation following concurrent training stimuli, definitive conclusions regarding the mechanisms relating to interference and the role of certain training variables still remain to be fully elucidated. In particular, clarity is sought regarding the existence and magnitude of the interference effect, and therefore the benefit of minimising this potential decline in strength and power parameters. Further research illuminating the recovery response to a concurrent stimulus of a resistance-endurance exercise order could prove particularly meaningful, for endurance athletes attempting to quantify the acute performance decrement following this exercise sequence.

As discussed, there are data to support and refute the existence of the interference phenomenon. Establishing a set of methods most likely to provide interference would be beneficial to the field. It is suggested that many of the training variables can be manipulated to exacerbate the interference of strength development. For example, an exercise mode of

running vs. cycling (Wilson et al., 2012), an increase in endurance session frequency (Jones et al., 2013), and reduced recovery between exercise modes (Robineau et al., 2016) have been reported to increase the likelihood of interference. Once established, it would be useful to observe whether these conditions do result in the observation of interference across a training study.

The origins of the interference phenomenon are set in the context of a training study (Hickson, 1980). To date, there is no strong evidence of an acute interference effect in human skeletal muscle. If an interference effect can be observed under conditions of greater construct validity; i.e. incorporating a condition of matched training load between concurrent and strength only training conditions (in addition to the original design), then it would seem appropriate to explore potential mechanisms in this context. In particular, it is suggested that an 'omics' approach would best facilitate progress in better understanding potential integrated molecular effectors provoking interference, with the underwhelming insights gleaned to date attributed to the complexity of cross-talk in human skeletal muscle (Coffey and Hawley, 2016).

A better understanding of the mechanisms implicated in a potential interference effect would be particularly interesting. Such information would ultimately help practitioners to manipulate variables to attenuate the interference. Neuromuscular mechanisms have been implicated in the interference effect (Cadore et al., 2013, Eklund et al., 2015). However, the seminal work in the field reported a divergence in strength performance at 7 wk (Hickson, 1980). It is suggested that the early improvements in strength performance are related to neuromuscular factors, with hypertrophy explaining the majority of subsequent improvements (Moritani and deVries, 1979).

Beyond mechanistic insight, clarity on the impact of training variables on recovery from and adaptation to concurrent training would be beneficial for practitioners. While this thesis has helped to better understand the role of exercise sequence in modifying strength

adaptation, the effect on power is still unknown. This is a pertinent question, given that power is suggested the outcome most susceptible to the interference phenomenon (Wilson et al., 2012). Finally, it would be of interest to understand the profile of recovery from strenuous concurrent exercise with a resistance-endurance exercise sequence. This observation might help practitioners to decide whether exercise order can be determined exclusively by training outcomes, or whether this must be considered in light of contrasting recovery permutations also.

## **8.7 Conclusions**

Research within this thesis has explored the acute and chronic responses to concurrent exercise. Whilst this has included an examination of the interference effect in part, the work has more broadly examined concurrent training from the perspective of optimising strength adaptation. Rather than adopt a myopic view of the concurrent training paradigm, whereby the addition of an endurance stimulus is fatal to strength-based training outcomes, the thesis has instead sought to better understand the response to a concurrent exercise stimulus and elucidate the response to manipulating training variables. This has been conducted with the observation of physiological parameters associated with the interference phenomenon i.e. strength and power, but also by observation of the early molecular responses to concurrent exercise stimuli. Research within this thesis has highlighted that while the acute training variable of exercise sequence might modify the adaptive response to concurrent training, this was not the case for the variable of endurance exercise intensity.

A primary conclusion of this thesis is that manipulating the order of exercise modes within a concurrent training paradigm is of significance; yet manipulating the intensity of the endurance exercise stimulus does not moderate the adaptive response. Amongst a much broader demographic of recreational exercisers, a resistance-endurance exercise order is

beneficial for improvements in lower-body dynamic strength, particularly so in individuals limited by time, such that they must train concurrently with minimal relief between modes of exercise. In contrast, observations relating to endurance exercise intensity are more specific to trained endurance athletes. Taken together, these findings indicate that practitioners programming for endurance athletes might be more concerned with intra-session exercise sequence, as opposed to the intensity of the endurance exercise stimulus, if attempting to elicit maximal strength adaptation within a concurrent training paradigm. Importantly, these findings are most relevant to settings whereby the athlete does not have the luxury of separating exercise modes, owing to constraints with training programming.

Beyond the role of training variables on the longer-term responses to concurrent stimuli, an isolated concurrent exercise session can have deleterious effects on subsequent training/performance capacity. Performance can be compromised for up to 48 h, even when the recovery process is supported by a habitual diet of good quality and protein supplementation. This highlights that when athletes must train concurrently with minimal relief between exercise modes, care must be employed regarding the proximity of subsequent sessions where high quality/intensity is required. This observation presents another important programming consideration for the practitioner. It is possible that an endurance-resistance exercise order might attenuate the muscle damage response, although this conclusion is somewhat speculative considering the specific research questions of this thesis. This question remains an interesting matter to address in the future, for those wishing to better understand the response from concurrent training stimuli. It is possible that exercise sequence be manipulated according to the competitive schedule i.e. adaptive *vs.* performance focus.

It appears that the early molecular responses to concurrent stimuli, specifically the mTOR signalling cascade, might offer limited insight in isolation into the adaptive response to concurrent exercise. This is indicated by the phosphorylation of mTOR and its primary effectors proving unmoved by concurrent exercise stimuli, which when repeated across a

training intervention, result in strength and power adaptation. Furthermore, these parameters were not sensitive to training status, as previously reported (Coffey et al., 2006). However, these observations relate to a limited time resolution in the early post-exercise response i.e. rest and 3 h. It is important to consider this in concluding the application of these findings. While a discord was observed between the acute molecular and chronic physiological responses to the exercise stimuli, this is not a unique finding. A more integrated approach, sympathetic to the complexities of regulatory processes within these molecular pathways, will likely prove more insightful. In particular, approaches with multiple outcomes aligned to post-transcriptional assessments, and indeed exercise adaptations observed through training studies, would assist in appraising such outcomes as a proxy for adaptation of human skeletal muscle and performance outcomes.

The research conducted as part of this thesis adds to the concurrent training literature. While there is evidence that concurrent training can result in the interference of strength *vs.* strength training in isolation, this should not impose a myopic view, whereby the combination of strength and endurance stimuli is fatal to strength adaptation. A concurrent training paradigm can elicit significant improvements in strength performance, particularly among endurance athletes. Furthermore, in situations where the expression of strength is important, certain acute training variables can be manipulated to optimise the strength training response within a concurrent training paradigm. To conclude, it is apparent that exercise sequence is an important variable to consider in programming, particularly so when constraints prohibit the separation of distinct exercise modes. Furthermore, this component might have implications for the quality of subsequent training/performance demands of close proximity to a strenuous concurrent training session. In contrast, it appears that endurance exercise intensity is of less significance, which will likely prove of important practical application to the endurance athlete and their support staff.

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