Adaptation to Concurrent Training: Role of Endurance Training Intensity

A thesis submitted in fulfilment of the requirements for the degree

Doctor of Philosophy

by

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Abstract

The simultaneous integration of both endurance and resistance exercise (RE) into a periodised training regime is termed concurrent training. As both exercise modes promote adaptations at both whole-body and skeletal muscle levels that improve oxidative as well as functional capacity, concurrent training is an attractive exercise strategy for improving markers of cardiometabolic health and athletic performance. Since the classic work of Hickson (1980), numerous investigations have shown that concurrent training, relative to resistance training (RT) performed alone, can attenuate improvements in maximal strength, hypertrophy, and indices of power development, with no negative impact on $\dot{V}O_{2max}$. This has been variously described as the interference effect or concurrent training effect. Despite the majority of the literature supporting the existence of the interference effect, some studies have not observed any evidence of an interference effect, or rather that some adaptations may be more susceptible to interference than others. The equivocal nature of this phenomenon suggests variations in the prescription of individual training variables may modulate the degree of interference seen with concurrent training. Identification of training variables mediating the interference effect will therefore allow for targeted exercise prescription to minimise interference during concurrent training.

Endurance training intensity is a particularly important practical consideration with concurrent training, given that high-intensity interval training (HIT) can be more effective for enhancing aerobic capacity, and for reducing cardiometabolic risk factors, compared with traditional moderate-intensity continuous training (MICT). Despite the efficacy of HIT for promoting positive health and performance outcomes, there is currently limited information on the effects of incorporating HIT into concurrent training programs when compared with MICT. There is evidence of interference to either maximal strength, power or hypertrophy development with concurrent training incorporating either HIT or MICT, or combinations of both. Consequently, the potential role of endurance training intensity in mediating any interference to RE adaptations remains unclear.

The primary aim of this thesis was to elucidate the potential role of endurance training intensity in mediating interference to exercise-induced anabolic responses in skeletal muscle and improvements in classical RT adaptations, compared with RT

performed alone. A thorough review of the current literature is presented in Chapter 2 of this thesis, followed by two experimental studies that directly address the primary aims of this thesis in Chapters 3-5. The focus of Chapter 3 (Study 1) was to investigate whether a single bout of concurrent exercise, incorporating either HIT or work-matched MICT cycling as the endurance training modality, differentially altered early post-exercise molecular responses involved in skeletal muscle hypertrophy compared to RE performed alone. Novel findings of this study were that skeletal muscle mTORC1 signalling was not compromised following subsequent RE compared with RE performed alone, despite similar metabolic perturbation induced in skeletal muscle by prior HIT or MICT. Rather, combining RE with HIT was a particularly potent stimulus for increasing mTOR and rps6 phosphorylation, and for reducing the expression of candidate microRNAs purported to negatively regulate the IGF-1/Akt, Fox-O1 and myogenesis signalling pathways in skeletal muscle.

Given the limitations of single-bout exercise studies for informing chronic skeletal muscle phenotypes induced by long-term exercise training, Chapters 4 and 5 (Study 2) of this thesis investigated exercise performance, morphological and molecular adaptations to eight weeks of concurrent training, incorporating either HIT or MICT as the endurance training modality, compared with RT performed alone. Major findings of this study were that compared with RT performed alone, concurrent training incorporating either HIT or work-matched MICT cycling similarly attenuated maximal lower-, but not upper-body strength development, while increases in lower-body lean mass were attenuated with concurrent training incorporating HIT, but not MICT. Training-induced improvements in selected counter-movement jump (CMJ) variables, including peak force and power, were also similarly attenuated following concurrent training incorporating HIT or MICT. These data corroborate existing evidence that endurance training can interfere with adaptations to RT, and extend current knowledge by suggesting endurance training intensity appears to not mediate interference to maximal strength gain, at least on a work-matched basis, while HIT may preferentially attenuate lean mass gain.

To provide further mechanistic insight into the adaptive responses to concurrent training, Chapter 5 of this thesis investigated skeletal muscle molecular adaptations to the training paradigm employed in Chapter 4. After completion of the training intervention, participants also underwent a group-specific single-bout exercise trial to quantify skeletal muscle molecular responses to exercise performed in a training-

accustomed state. Supporting the lean mass responses to training observed in Chapter 4, concurrent training incorporating HIT, but not MICT, attenuated the training-induced increase in type I muscle fibre cross-sectional area (CSA) relative to RT performed alone. In contrast to Chapter 3, whereby exercise was performed in a relatively training-unaccustomed state, a single bout of post-training RE preferentially induced mTORC1 signalling and the phosphorylation of key regulators of ribosome biogenesis in skeletal muscle compared with concurrent exercise. Despite these divergent upstream signalling responses, little evidence of ribosome biogenesis adaptation in skeletal muscle was observed in the basal state following RT, while changes in both the 45S ribosomal RNA (rRNA) precursor and the mature ribosomal rRNAs 5.8S and 28S were greater with concurrent exercise, mirroring changes in total RNA content of skeletal muscle. These data suggest concurrent training is a more potent stimulus for inducing adaptations to translational capacity in skeletal muscle compared with single-mode RT, at least after eight weeks of training, and that these mechanisms do not appear to explain interference to maximal strength gain or markers of muscle hypertrophy.

This thesis has extended current knowledge of the molecular basis of interference between concurrent resistance and endurance exercise, and the role of endurance training intensity in mediating the interference effect. The findings of this thesis further question interference to mTORC1 signalling as a mechanism for attenuated muscle hypertrophy and maximal strength during concurrent training, and present novel data information suggesting adaptations to translational capacity in skeletal muscle are also not compromised with short-term concurrent training compared with single-mode RT. In a practical sense, this thesis highlights that incorporating either HIT or MICT into a concurrent training program does not modulate interference to maximal strength gain, at least on a work-matched basis, although HIT may compromise lean mass gain, mediated largely via attenuated type I muscle fibre CSA. Either HIT or MICT may therefore be successfully incorporated into concurrent training programs where the goal is increased maximal strength as well as improved aerobic capacity. The work presented in this thesis provides a platform for future work to investigate the role of additional training variables in the interference effect, as well as novel molecular targets warranted for further investigation as potential markers of interference with long-term concurrent training.

Declaration

I, Mr Jackson Fyfe, declare that the PhD thesis entitled “Adaptation to Concurrent Training: Role of Endurance Training Intensity” is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signature: Date:

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Publications arising from this thesis

1. **Fyfe, J.J.,** Bishop, D.J., & Stepto, N.K. (2014). Interference between Concurrent Resistance and Endurance Exercise: Molecular Bases and the Role of Individual Training Variables. *Sports Medicine, 44*(6), 743-762.

2. **Fyfe, J.J.,** Bishop, D.J., Zacharewicz, E., Russell, A.P., & Stepto, N.K. (2016). Concurrent exercise incorporating high-intensity interval or continuous training modulates mTORC1 signalling and microRNA expression in human skeletal muscle. *American Journal of Physiology, Regulatory Integrative and Comparitive Physiology*. Jun 1;310(11):R1297-311.

3. **Fyfe, J.J.,** Bartlett, J.D., Hanson, E.D., Stepto, N.K., & Bishop, D.J. (2016). Endurance training intensity and interference during concurrent training. *Frontiers in Physiology*. (In review).

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Research grants arising from this thesis

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3. **Fyfe, J.J.,** Bartlett, J.D., Hanson, E.D., Anderson, M.J., Garnham, A.P., Bishop, D.J., & Stepto, N.K. (2016). Ribosome biogenesis in human skeletal muscle following concurrent training compared with single-mode resistance training. *European College of Sport Science (ECSS) annual meeting, Vienna, Austria.*

4. **Fyfe, J.J.** (2016). Interference during concurrent training: potential mechanisms and the role of individual training variables (Invited presentation). *Exercise and Sport Science Australia (ESSA) annual meeting, Melbourne, Australia.*

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List of Abbreviations

µg	microgram
µL	microlitre
µM	micromolar
µm	micrometre (micron)
1-RM	one-repetition maximum
4E-BP1	eukaryotic initiation factor 4E binding protein 1
5'-TOP	5'-tract of pyrimidine
ACC	acetyl-coenzyme A carboxylase
Acetyl-CoA	acetyl coenzyme A
AE	aerobic exercise
AID	autoinhibitory domain
AMP	adenosine monophosphate
AMPK	5' adenosine monophosphate-activated protein kinase
AMPKK	5' adenosine monophosphate-activated protein kinase kinase
ANOVA	analysis of variance
AS160	Akt substrate of 160 kilodaltons
Atg	autophagy-specific gene
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BCP	1-bromo-3-chloropropane
Ca^{2+}	calcium ion
CaMKII	calcium/calmodulin-dependent kinase II
CaMKK	calcium/calmodulin-dependent kinase kinase
cAMP	cyclic adenosine monophosphate
CBS	cystathionine β-synthase
CDK	cyclin-dependent kinase
CE	concurrent exercise
CHO	carbohydrate
CL	confidence limit
CMJ	counter-movement jump
CO_2	carbon dioxide

CSA	cross-sectional area
CV	coefficient of variation
d	Cohen's standardised effect size
Deptor	dishevelled, eg1-10, pleckstrin domain protein interacting with mTOR
DTT	DL-dithiothreitol
EAA	essential amino acid
EDTA	ethylenediaminetetraacetic acid
eEF2K	eukaryotic elongation factor 2 kinase
eEF	eukaryotic elongation factor
EE	endurance exercise
EGTA	ethyleneglycol-bis-(*β*-aminoethylether)-*N,N,N',N'*-tetraacetic acid
eIF	eukaryotic initiation factor
eNOS	nitric oxide synthase
eRF	eukaryotic release factor
ERK 1/2	extracellular signal-regulated kinase 1/2
ET	endurance training
ES	effect size
Fox-O1	forkhead box-O1
Fox-O3a	forkhead box-O3a
FSR	fractional synthesis rate
GβL	g-beta-L protein
GAP	guanosine triphosphate activating protein
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GATOR1	guanosine triphosphatase-activating protein activity towards Rags 1
GATOR1	guanosine triphosphatase-activating protein activity towards Rags 2
GDP	guanosine diphosphate
GEF	guanine nucleotide exchange factor
GLUT4	glucose transporter isoform 4
GSK-3α	glycogen synthase kinase-3 alpha isoform
GSK-3β	glycogen synthase kinase-3 beta isoform
GTP	guanosine triphosphate
GTPase	guanosine triphosphatase
HDAC5	histone deacetylase 5
h	hour

H^+	hydrogen ion, proton
HCl	hydrochloric acid
HIF-1α	hypoxia inducible factor-1α
HIT	high-intensity interval training
HRR	heart rate reserve
IGF-1	insulin-like growth factor
IKKβ	inhibitor of nuclear factor kappa-B kinase subunit beta
IL	interleukin
IRS	insulin receptor substrate
kDa	kilodalton
kg	kilogram
kJ	kilojoule
L	litre
LAMTOR	late endosomal/lysosomal adaptor, MAPK and mTOR activator
LKB1	liver kinase B1
LT	lactate threshold
m	metre
M	molar
MaFbx	muscle atrophy F-box
MAPKAP1	mammalian stress-activated protein kinase interacting protein 1
MAPK	mitogen-activated protein kinase
Met-tRNA	initiator transfer ribonucleic acid
Mg^{2+}	magnesium ion
MGF	mechano-growth factor
miRNA	micro ribonucleic acid
MICT	moderate-intensity continuous training
min	minute
mL	millilitre
mLST8	mammalian lethal with SEC13 protein 8
mM	millimolar
mmol	millimole
MPB	muscle protein breakdown
MPS	muscle protein synthesis
MRI	magnetic resonance imaging

mRNA	messenger ribonucleic acid
mTOR	mechanistic target of rapamycin
mTORC1	mechanistic target of rapamycin complex 1
mTORC2	mechanistic target of rapamycin complex 2
MuRF-1	muscle RING-finger 1
MyoD	myogenic differentiation 1
N	newton
Na_3VO_4	sodium orthovanadate
Na^+	sodium ion
NaCl	sodium chloride
NAD^+	oxidised nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NAD^+:NADH	oxidised nicotinamide adenine dinucleotide: reduced NAD^+ ratio
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NaF	sodium flouride
NO	nitric oxide
NaOH	sodium hydroxide
Nm	newton-metre
O_2	oxygen
p70S6K1	70 kilodalton ribosomal protein subunit kinase 1
p70S6K2	70 kilodalton ribosomal protein subunit kinase 2
PABP	poly(A) binding protein
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDCD4	programmed cell death protein 4
PDK-1	phosphoinositol-dependent kinase-1
PDK	pyruvate-dehydrogenase kinase
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator-1α
pH	negative logarithm (base 10) hydrogen ion activity/concentration
PI3K	phosphatidylinositol 3-kinase
PIP2	$PI4,5P_2$
PIP3	$PI4,5P_3$
PKB	protein kinase B
PMSF	phenylmethanesulfonyl flouride

PP2C	protein phosphatase 2C
PRAS40	proline-rich Akt substrate of 40 kilodaltons
PRR5	proline rich 5 (renal)
PVDF	polyvinylidine fluoride
Rag	ras-related guanosine triphosphate binding
Raptor	regulatory associated protein of mTOR
rDNA	ribosomal deoxyribonucleic acid
RE	resistance exercise
REDD1	regulated in deoxyribonucleic acid damage and development 1
RFD	rate of force development
Rheb	ras homolog enriched in brain
Rictor	rapamycin-insensitive companion of mammalian target of rapamycin
RM	repeated measures
RNA	ribonucleic acid
RPE	rating of perceived exertion
rpm	revolutions per minute
rps6	ribosomal protein S6
rRNA	ribosomal ribonucleic acid
RSA	repeated-sprint ability
RT	resistance training
s	second
SD	standard deviation
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
Ser	serine
SIRT	sirtuin
SIT	sprint-interval training
SL-1	selectivity factor-1
TAK1	transforming growth factor β-activated kinase 1
TBC1D1	TBC1 domain family member 1
TBC1D4	TBC1 domain family member 4
TBS	Tris-buffered saline
TBST	Tris-buffered saline with Tween-20
Thr	threonine

TIF-1A	transcription initiation factor 1A
TNFα	tumour necrosis factor alpha
tRNA	transfer ribonucleic acid
TSC1	tuberous sclerosis complex 1
TSC2	tuberous sclerosis complex 2
UBF	upstream binding factor
ULK-1	Unc-51-like kinase 1
UTR	5'-untranslated region
V-ATPase	vacuolar H+-adenosine triphosphatase
VEGF	vascular endothelial growth factor
$\dot{V}O_2$	volume of oxygen uptake (per minute)
$\dot{V}O_{2max/peak}$	maximal/peak volume of oxygen uptake (per minute)
W	watt
wk	week
W_{peak}	peak aerobic power
y	year

Chapter 1. General introduction

Skeletal muscle is a highly malleable tissue capable of significant metabolic and morphological adaptations in response to disruptions in cellular homeostasis, such as during exercise (Coffey & Hawley, 2007; Fluck & Hoppeler, 2003). Endurance and resistance training (RT) represent divergent exercise modes, with each inducing distinct responses within the muscle milieu that act to minimise cellular stress during subsequent exercise bouts (Hawley, 2009). In this regard, the skeletal muscle adaptations associated with exercise training are highly specific to the mode of exercise performed (i.e., resistance [RE] vs. endurance exercise), along with the frequency, intensity and duration of the exercise stimulus (Hawley, 2002). For example, long-term RT promotes enhanced muscle activation and fibre hypertrophy, resulting in increased maximal contractile force (Folland & Williams, 2007; Tesch, 1988). Conversely, long-term endurance training increases mitochondrial density and oxidative capacity of the trained muscle fibres (Holloszy, 1967), and promotes alterations in substrate metabolism (Holloszy & Coyle, 1984), culminating in increased whole-body aerobic capacity ($\dot{V}O_{2max}$) (Hawley, 2002).

Concurrent training can be defined as the simultaneous integration of endurance and RE into a periodised training regime (Leveritt et al., 1999). Despite the wide-ranging benefits of combining these divergent exercise modes, there is now considerable evidence concurrent training can compromise the development of muscle mass, strength and indices of power or rate of force development (RFD) compared with undertaking RE alone (Hickson, 1980; Leveritt et al., 1999; Wilson et al., 2012). This phenomenon has been variously described as the interference effect or concurrent training effect (Hawley, 2009; Leveritt et al., 1999). Current global health guidelines recommend a combination of resistance and endurance exercise be performed to counteract metabolic disease and declines in aerobic capacity occurring across the lifespan (Garber et al., 2011; Haskell et al., 2007). Concurrent training is also essential for athletes requiring elements of maximal strength, power, and hypertrophy in combination with a high aerobic capacity (Helgerud et al., 2011; Nader, 2006). Despite the potential for compromised adaptation with concurrent training, current exercise guidelines (Garber et al., 2011; Haskell et al., 2007) fail to address practical strategies to mitigate the interference effect.

Further insight into the mechanisms responsible for the interference phenomenon is required to inform practical guidelines aimed at minimising the interference effect (Fyfe et al., 2014). While these mechanisms are likely to be

multifactorial, endurance exercise presumably either interferes with the 'quality' of RE sessions (via residual fatigue and/or substrate depletion) (Leveritt et al., 1999), and/or compromises the early molecular responses activated by RE mediating transient post-exercise increases in muscle protein synthesis (MPS) and subsequently muscle fibre hypertrophy (Baar, 2006; Hawley, 2009; Nader, 2006). In recent years, insight into the molecular factors mediating the specific adaptations to divergent exercise stimuli has emerged. This has in turn provided insight into the potential molecular bases for the interference effect in skeletal muscle. Training adaptations in skeletal muscle are generally considered to be the cumulative result of early signalling responses and ensuing gene expression initiated after repeated exercise bouts, resulting in the accumulation of specific proteins over time and, subsequently, an altered muscle phenotype (Egan et al., 2013; Perry et al., 2010). The mechanistic (formerly mammalian) target of rapamycin complex 1 (mTORC1) pathway has been identified as a key mediator of load-induced increases in MPS and subsequently muscle growth (Bodine et al., 2001b; Drummond et al., 2009), whereas the 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK) and Ca^{2+}/calmodulin-dependent kinase II (CaMKII) cascades, among others, are activated by endurance exercise and converge on the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) to coordinate mitochondrial biogenesis and other metabolic adaptations in skeletal muscle (McGee & Hargreaves, 2010; Wu et al., 2002). Previous work in rodent skeletal muscle (Atherton et al., 2005) suggested divergent signalling responses activated by RE and endurance exercise-like electrical stimulation may explain the divergent adaptations to chronic endurance and RT. Various molecular responses considered to be predominantly induced in skeletal muscle by endurance exercise, including activation of the AMPK pathway, can negatively regulate mTORC1 signalling and protein synthesis both in rodent skeletal muscle and *in vitro* (Atherton et al., 2005; Bolster et al., 2002; Inoki et al., 2003a). Subsequent work in humans (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015) has focused on the hypothesis that attenuated muscle hypertrophy with concurrent training (Bell et al., 2000; Kraemer et al., 1995; Wilson et al., 2012) may be explained by AMPK-mediated inhibition of the mTORC1 pathway. Human studies have, however, shown less specificity in early signalling responses to single bouts of endurance and resistance exercise in skeletal muscle

(Camera et al., 2010; Coffey et al., 2006a; Coffey et al., 2006b; Vissing et al., 2011; Wilkinson et al., 2008). Moreover, several studies have demonstrated single bouts of concurrent exercise do not compromise either mTORC1 signalling or rates of MPS (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Donges et al., 2012; Pugh et al., 2015), or rather potentiates these responses (Lundberg et al., 2012), compared with RE performed alone. It is therefore unclear whether endurance exercise-induced attenuation of mTORC1 signalling and MPS in human skeletal muscle, or potentially other mechanisms, mediate the concurrent interference effect. Novel molecular mechanisms with the potential to regulate adaptations to exercise in skeletal muscle have recently emerged, including altered miRNA expression (Hitachi & Tsuchida, 2013; Russell et al., 2013; Zacharewicz et al., 2013) and ribosome biogenesis (Chaillou et al., 2014; Figueiredo et al., 2015). To date, these mechanisms have not been investigated in the context of concurrent training, and their potential contribution to altered skeletal muscle adaptation during concurrent training remains unknown.

From a practical standpoint, elucidation of the roles of specific training variables in the interference effect is required to inform exercise prescription aimed at minimising interference during concurrent training (Fyfe et al., 2014). However, given the multitude of potential concurrent training variables (e.g., endurance and RE order, length of between-mode recovery, endurance training volume, intensity and modality), the roles of these variables in the interference effect are not fully understood (Fyfe et al., 2014). One practical consideration is the intensity of endurance training employed in a concurrent training regime. There has been increased interest in the efficacy of high-intensity interval training (HIT) compared with traditional moderate-intensity continuous training (MICT) for improving cardiometabolic risk factors (Gibala et al., 2012; Hawley & Gibala, 2012; Weston et al., 2014a) and indices of aerobic exercise performance (Milanovic et al., 2015; Weston et al., 2014b). When compared with work-matched MICT, HIT protocols (e.g., 2-4 min work intervals interspersed with 1-3 min of active or passive recovery), can be more effective for improving indices of exercise performance including $\dot{V}O_{2max}$ (Gormley et al., 2008; Milanovic et al., 2015; Weston et al., 2014b) and repeated-sprint ability (Edge et al., 2005), are well-tolerated in clinical populations (Tjonna et al., 2008; Wisloff et al., 2007), and perceived as more enjoyable despite eliciting higher ratings of perceived exertion (Bartlett et al., 2011). The efficacy of HIT compared with traditional MICT has been attributed, at least in part, to its potency for inducing molecular responses in skeletal muscle related to mitochondrial

biogenesis (e.g., AMPK activation and PGC-1α expression) and enhanced oxidative capacity (Burgomaster et al., 2008; Gibala et al., 2012; Gibala et al., 2009).

Despite the relevance of high-intensity exercise for improving markers of aerobic exercise performance and metabolic health, in addition to its implications for exercise enjoyment and adherence, little attention has been paid to the effect of incorporating either HIT or MICT into concurrent training programs. Compromised adaptations to maximal strength, hypertrophy, or indices of power development have been reported in concurrent training studies incorporating HIT (Chtara et al., 2008; Dudley & Djamil, 1985; Kraemer et al., 1995), MICT (Craig et al., 1991; Gergley, 2009), or combinations of both (Bell et al., 2000; Hakkinen et al., 2003; Hickson, 1980). Only a single study has to date compared concurrent training groups performing endurance exercise of different endurance training intensities (Silva et al., 2012). Higher-intensity endurance exercise is associated with exacerbated residual fatigue (Bentley et al., 2000; de Souza et al., 2007) of the exercised musculature, and also induces greater AMPK activation in skeletal muscle (Rose et al., 2009b), when compared to lower-intensity continuous exercise. It is therefore unclear whether HIT represents a more favourable exercise strategy, when compared with MICT, from the perspective of limiting interference to RE adaptations when incorporated into concurrent training programs.

The review of literature for this thesis (Chapter 2) will firstly discuss the concepts of the specificity of training adaptation and interference between concurrent endurance and RE. The regulatory processes governing protein synthesis will then be introduced, to form a platform upon which the potential molecular mechanisms underlying the specificity of training adaptation and concurrent interference effect will be explored. A final section of the review of literature (expanded and updated from our published review article; Fyfe et al. (2014)) will then discuss the potential role of specific concurrent training variables in the interference effect, along with the limitations of our current understanding of this complex paradigm. A particular focus of this thesis will be examining the potential role of endurance training intensity, using HIT and work-matched MICT as a comparative exercise model, in modulating the interference effect during concurrent training. Chapters 3 (Study 1) and 5 (Study 2) will examine the early molecular events occurring in human skeletal muscle following single bouts of concurrent exercise incorporating either HIT or MICT as the endurance exercise modality. Specifically, Chapter 3 will examine perturbations in mTORC1

signalling and the expression of microRNA species implicated in skeletal muscle adaptations to exercise, while Chapter 5 will explore the regulation of ribosome biogenesis, as well as mTORC1 signalling, both at rest after 8 weeks of concurrent training and following a single post-training exercise bout. Owing to the limitations to single-bout exercise studies for extrapolating the long-term efficacy of these protocols on chronic training adaptations, Chapter 4 (Study 2) will explore the effects of 8 weeks of concurrent training, incorporating either HIT or MICT, on exercise performance and morphological adaptations when compared with RT performed alone.

Together, this thesis aims to enhance the body of knowledge on whether endurance training intensity plays a role in mediating interference to i) molecular responses in skeletal muscle purported to play a role in mediating muscle fibre hypertrophy, and ii) adaptations consequent to RT including muscle hypertrophy, maximal strength, and indices of power development, relative to RT performed alone. It is anticipated the findings of this thesis will inform practical recommendations for the prescription of HIT or MICT for minimising interference to RT adaptations during periods of concurrent training.

Chapter 2. Literature review

Adapted from: Fyfe, J. J., Bishop, D. J., & Stepto, N. K. (2014). Interference between Concurrent Resistance and Endurance Exercise: Molecular Bases and the Role of Individual Training Variables. *Sports Medicine*, *44*(6), 743-762.

2.1 Literature search

The articles selected for review were obtained via searches of MEDLINE and SPORTDiscus^{TM} between 1957 and February 2016. The following keywords were searched in combination: 'concurrent training', 'molecular', 'interference' 'protein synthesis', 'mitochondrial biogenesis', 'exercise', 'HIT', 'continuous' and 'training adaptation'. From the abstracts returned, articles were included for review if they related to the molecular basis for the specificity of training adaptation, the molecular regulation of skeletal muscle protein synthesis, or interference associated with concurrent versus single-mode training. Literature cited in each article chosen was also searched, and additional articles satisfying the above criteria were likewise included for review.

2.2 The specificity of training adaptation

Resistance and endurance exercise represent divergent exercise modes, both with regards to their inherent stimuli and the subsequent training-induced adaptations induced within skeletal muscle. For example, traditional continuous-style endurance exercise involves low-intensity, prolonged-duration contractile activity, whereas typical RE is characterised by relatively high-intensity and short-duration muscular contractions. It follows that the skeletal muscle adaptations induced by long-term training are highly specific to the mode of exercise performed (Hawley, 2009). The principal adaptation in skeletal muscle to long-term RE is muscle fibre hypertrophy (Fry, 2004), which is the cumulative result of transient increases in net MPS relative to muscle protein breakdown (MPB) occurring for 24-48 hours post-exercise (Atherton & Smith, 2012; Phillips et al., 1997). In contrast, endurance exercise is generally characterised by lower intensity, longer-duration contractile activity, imparting far less mechanical strain on the active muscle fibres compared with typical RE (Baar, 2009). This presents a significant metabolic challenge within the muscle milieu, causing perturbations in intracellular concentrations of Ca^{2+}, oxygen, lactate, reactive oxygen species (ROS), and increased AMP:ATP (adenosine triphosphate) and NAD^{+}:NADH (nicotinamide adenine dinucleotide: NAD^{+} reduced form) ratios (Coffey & Hawley, 2007). These stimuli promote skeletal muscle adaptations primarily associated with enhanced oxidative capacity, including mitochondrial biogenesis (Baar et al., 2002;

Pilegaard et al., 2003; Wu et al., 2002), improved substrate utilisation (Holloszy & Coyle, 1984) and enhanced capillary density (Saltin & Gollnick, 1983). It should be noted, however, that although skeletal muscle adaptations to exercise are highly mode-specific, some degree of crossover in the specificity of these adaptations exists. For example, aerobic exercise training is capable of inducing modest skeletal muscle hypertrophy (Konopka & Harber, 2014), while RE can promote mitochondrial biogenesis and enhanced oxidative capacity in skeletal muscle (Salvadego et al., 2013). Nevertheless, it appears likely that exercise-mode-specific adaptations accumulate over time, as evidenced by the divergent phenotypes of highly-trained strength and endurance athletes (Coffey et al., 2006b).

2.3 Concurrent training and the interference effect

Simultaneously integrating both resistance and endurance exercise within a periodised training regime is termed concurrent training (Leveritt et al., 1999). As both exercise modes can promote adaptations that counteract a number of disorders impacting upon functional capacity and metabolic health, including sarcopenia (Evans, 1995; Pijnappels et al., 2008; Reeves et al., 2004), type II diabetes, and obesity (Kelley et al., 2002; Kelley et al., 1996; Morino et al., 2005), concurrent training is an attractive exercise strategy for counteracting multiple disease states. Current global health guidelines indeed recommend a combination of resistance and endurance exercise be performed to counteract the decline in physical capacity occurring across the lifespan (Garber et al., 2011; Haskell et al., 2007). Additionally, from an athletic perspective, concurrent training is necessary for athletes requiring the development of combinations of maximal strength, power and muscle hypertrophy, concomitantly with a high aerobic capacity (Helgerud et al., 2011).

Despite the obvious benefits of combining resistance and endurance exercise, the classic work of Hickson (1980) first established that concurrent training can results in compromised adaptation compared with training for either exercise mode alone (Leveritt et al., 1999). This phenomenon has been variously described as the interference effect or concurrent training effect (Hawley, 2009; Wilson et al., 2012). This interference effect typically manifests as a compromised RT adaptations relative to RT undertaken alone. For example, concurrent training can compromise maximal

strength (Bell et al., 2000; Dolezal & Potteiger, 1998; Hakkinen et al., 2003; Hennessy & Watson, 1994; Hickson, 1980; Kraemer et al., 1995), muscle hypertrophy (Bell et al., 2000; Kraemer et al., 1995) and indices of power development (Hakkinen et al., 2003; Hennessy & Watson, 1994; Hunter et al., 1987; Kraemer et al., 1995; Leveritt & Abernethy, 1999) compared with RT performed alone. Conversely, RT appears to have minimal to no negative impact on endurance performance and $\dot{V}O_{2max}$ (Leveritt et al., 1999; Wilson et al., 2012), although compromised aerobic capacity development has been reported with concurrent training compared to endurance training alone (Nelson et al., 1990). Rather, than compromising endurance capacity, concurrent training can augment both short- (<15 min) and longer-duration (>30 min) endurance performance, predominantly via improvements in neuromuscular function and economy (Aagaard & Andersen, 2010; Aagaard et al., 2011). It should also be noted, however, that not all concurrent training studies show any interference effect to either muscle hypertrophy, strength, or power development (Balabinis et al., 2003; McCarthy et al., 2002; Sillanpaa et al., 2009). Importantly, these equivocal findings are likely mediated by differences in individual training variables employed in each concurrent training study, in addition to the training and nutritional status of participants (Fyfe et al., 2014), which will be further discussed in later sections of this review. Given muscle fibre hypertrophy is considered the primary adaptation induced by long-term RE (Folland & Williams, 2007; Fry, 2004; Tesch, 1988), and concurrent training can attenuate muscle hypertrophy compared with RE performed alone (Bell et al., 2000; Kraemer et al., 1995), the following section will discuss the regulation of skeletal muscle hypertrophy consequent to RE.

2.4 Regulation of skeletal muscle hypertrophy

The accretion of skeletal muscle mass (i.e., muscle hypertrophy) is the primary adaptation induced in skeletal muscle by long-term RE (Folland & Williams, 2007; Fry, 2004; Tesch, 1988). In humans, increased whole-muscle cross-sectional area (CSA) is believed to occur via increases in the CSA of individual muscle fibres (i.e., muscle fibre hypertrophy) as opposed to increased muscle fibre number (i.e., muscle fibre hyperplasia) (Folland & Williams, 2007). Increased fibre CSA reflects an increased abundance of contractile material (i.e., cross-bridges) arranged in-parallel within each

myofibre, facilitating an increase in force-generating capacity (Folland & Williams, 2007). Changes in muscle fibre CSA are consequent to the dynamic nature of protein turnover in skeletal muscle, and result from transient perturbations in rates of MPS, MPB, or both (Phillips et al., 1997; Rasmussen & Phillips, 2003). In healthy, recreationally-active individuals, muscle protein turnover rates are ~1.2% per day, and exist in a dynamic equilibrium whereby MPS exceeds MPB in the fed state, and MPB exceeds MPS in the fasted state, the net effect being maintenance of skeletal muscle mass in the absence of additional anabolic or catabolic stimuli. Persistent elevations in rates of MPS relative to MPB results in an accrual of skeletal muscle protein and subsequently fibre hypertrophy, whereas muscle fibre atrophy occurs when rates of MPB are sustained above MPS (Phillips et al., 1997). It is therefore the net balance of protein turnover, sustained over time, which determines the resultant change in muscle fibre CSA.

Exercise and nutrients, particularly essential amino acids (EAAs), are independent regulators of MPS (Atherton & Smith, 2012). After a single bout of exercise, an initial latency period occurs during which there are no measurable increases in MPS, which appears to be related to the degree of energy/mechanical stress induced by the exercise stimulus (Atherton & Smith, 2012). For example, MPS is supressed in rodent skeletal muscle during muscular contraction in a work-dependent manner (Atherton & Rennie, 2006; Rose et al., 2009a), while in humans MPS is supressed for >3 h after highly-fatiguing and damaging eccentric contractions (Cuthbertson et al., 2006), and for <1 h after lower-intensity RE (Kumar et al., 2009). After this initial latency period, MPS rises sharply between 45 and 150 min and can be sustained for up to 4 h post-exercise in the fasted state (i.e., limited by substrate availability) (Kumar et al., 2009). With the provision of amino acids, MPS can be sustained for at least 24 h post-exercise in human skeletal muscle (Churchward-Venne et al., 2012; Cuthbertson et al., 2006) (Figure 2.1). As well as stimulating increased MPS, RE also induces a significant but smaller increase in rates of mixed MPB, which remains elevated for at least 24 h post-exercise (Phillips et al., 1997). Deducing the critical stimuli and molecular mechanisms that mediate alterations in MPS and degradation induced by RE are therefore essential to understanding the regulation of chronic adaptations to RE in human skeletal muscle. Moreover, such information may improve understanding of how these pathways become deregulated in disease states characterised by significant muscle wasting (e.g., sarcopenia, sepsis, and cancer cachexia).

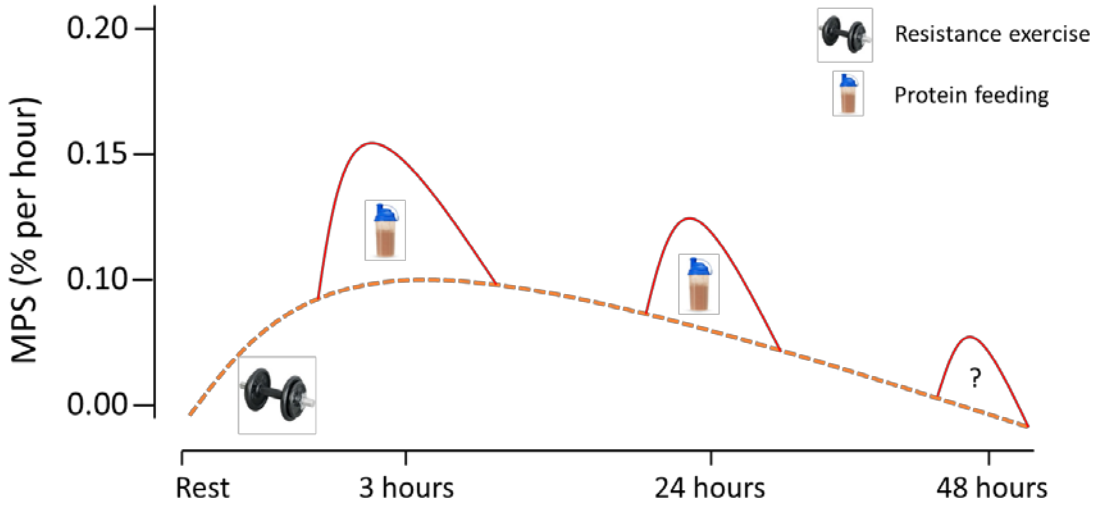

Figure 2.1 Time course of the muscle protein synthesis (MPS) response to a single bout of resistance exercise. With the addition of amino acid provision, MPS can remain elevated for >24 h post-exercise. Adapted from Churchward-Venne et al. (2012).

In addition to increases in contractile protein content, increases in muscle fibre CSA are also associated with the incorporation of new myonuclei originating from satellite cell populations (Mauro, 1961; Pallafacchina et al., 2013). Satellite cells are undifferentiated stem cells lying dormant beneath the basal lamina surrounding each myofibre. Upon activation, satellite cells are incorporated as new nuclei within the muscle fibre and can then contribute to the production of new contractile proteins (Ten Broek et al., 2010). However, the relative contributions of myogenesis from satellite cell recruitment and increases in MPS to load-induced skeletal muscle hypertrophy in mature muscle fibres is still hotly debated (O'Connor & Pavlath, 2007; Rehfeldt, 2007). Observations of a maintained ratio between nuclear and cytoplasmic material in a variety of muscle growth models (Burleigh, 1977; Eisenberg et al., 1989) and correlations between fibre size and myonuclei number (Landing et al., 1974) support the notion that satellite cell recruitment occurs concomitant with increases in muscle fibre size. However, whether satellite cell recruitment is obligatory for muscle fibre hypertrophy remains controversial, as robust fibre hypertrophy is possible, albeit in mouse skeletal muscle, despite considerable (>90%) satellite cell depletion (McCarthy et al., 2011). As skeletal muscle hypertrophy consequent to long-term RE is considered primarily mediated by dynamic post-exercise perturbations in MPS (Glynn et al., 2010;

Phillips et al., 1997), the following section will discuss the molecular regulation of protein synthesis.

2.5 Molecular regulation of skeletal muscle protein synthesis

Before discussing the potential molecular mechanisms underlying interference to MPS and skeletal muscle hypertrophy with concurrent training, an understanding of the regulation of protein synthesis is necessary. Protein synthesis involves the translation of messenger RNA (mRNA) transcripts at the ribosome and the subsequent incorporation of individual amino acids into a nascent peptide chain (Kapp & Lorsch, 2004). Understanding the molecular mechanics of mRNA translation, as well as the upstream signalling pathways regulating the activity of the translational machinery, is therefore key to understanding the regulation of protein synthesis in skeletal muscle.

2.5.1 Regulatory steps of skeletal muscle protein synthesis

2.5.1.1 Protein translation initiation, elongation and termination

The process of mRNA translation is characterised by a series of reactions that can be functionally divided into three phases: initiation, elongation and termination (Gordon et al., 2013; Kapp & Lorsch, 2004). Protein translation is predominantly regulated at the initiation step, with much less regulation occurring at either the elongation or termination steps (Kapp & Lorsch, 2004). It follows that translation initiation is a complex process, requiring the contribution of at least 12 initiation factors, many of which are known downstream targets of regulatory signalling pathways (Hinnebusch & Lorsch, 2012). The first step in translation initiation involves formation of the ternary complex, comprising the eukaryotic initiation factor 2 (eIF2), initiator Met-tRNA (transfer RNA) and GTP (guanosine triphosphate). Once formed, the ternary complex then associates with other initiation factors, including eIFs 1, 1A, 5 and the eIF3 complex, forming a multifactor complex that binds the 40S ribosomal subunit to form the 43S pre-initiation complex (Hinnebusch & Lorsch, 2012; Kapp & Lorsch, 2004). The next translational step involves binding of the 43S pre-initiation complex to the mRNA near the 5’7-methylguanosine cap (Hinnebusch & Lorsch, 2012). The eIF4F complex assembles on the 5’ cap of the mRNA and begins unwinding structures found on the 5’-untranslated region (UTR), assisted by other initiation factors including

eIF4A, eIF4B and eIF4H. eIF4F, in conjunction with eIF3 and the poly(A) binding protein (PABP) bound to the 3'-poly(A) tail of the mRNA, then loads the mRNA onto the 43S pre-initiation complex. Once bound near the cap, the 43S complex then scans along the mRNA in search of the AUG initiator codon. After identification of the initiator codon, base-pairing occurs between the mRNA and the Met-tRNA anticodon in the peptidyl-tRNA (P) site of the 40S subunit. This initiates the hydrolysis of GTP from eIF2 and subsequent release of eIF2-GDP (guanosine diphosphate) from the pre-initiation complex. To permit efficient recycling of eIF2 and reformation of the ternary complex, the GDP bound to eIF2 must be re-exchanged for GTP. This GDP-GTP exchange is mediated by eIF2B, of which eIF2Bε appears to be the predominant subunit involved (Reid et al., 2012). Once GTP is hydrolysed from eIF2, eIF2-GDP then releases the Met-tRNA into the P site before disassociating from the complex, along with most of the other initiation factors (Gordon et al., 2013). eIF5B then facilitates the recruitment of the large (60S) ribosomal subunit to the 40S-Met-tRNA-mRNA complex (Pestova et al., 2000), forming the 80S initiation complex now functionally competent to proceed with peptide elongation (Gordon et al., 2013). The subsequent GTP hydrolysis of eIF5B promotes its disassociation from the 80S complex, which is considered the final step in translation initiation (Gordon et al., 2013).

The elongation phase of protein synthesis involves polypeptide assembly at the ribosome, a process requiring a considerable amount of cellular energy (Browne & Proud, 2002; Kaul et al., 2011). Peptide-chain elongation is mediated by a number of non-ribosomal proteins designated as eukaryotic elongation factors (eEFs). This process occurs in two phases, the first involving recruitment of amino-acyl tRNAs to the A-site of the ribosome, mediated by eEF1A, and the second involving translocation of the ribosome along the mRNA, facilitated by the eukaryotic elongation factor 2 (eEF2) (Browne & Proud, 2002). Elongation begins with a peptidyl tRNA located in the ribosomal P-site next to a vacant-A site. An aminoacyl tRNA is then transported to the ribosomal A-site as part of a ternary complex with eEF1A and GTP. Codon-anticodon base pairing occurs between the mRNA and tRNA, which induces three bases in the small ribosomal subunit's rRNA to interact with the resultant mRNA-tRNA duplex. This appears to activate eEF1A's GTPase activity, and eEF1A-GDP then releases the amino-acyl tRNA into the ribosomal A-site to proceed with peptide bond formation. The formation of the peptide bond between the incoming amino acid and the peptidyl tRNA is catalysed by the ribosomal peptidyl transferase center (Moore & Steitz, 2003).

This results in a deacetylated tRNA in a hybrid state, with its acceptor end in the exit (E) site of the large ribosomal subunit and its anticodon end in the P site of the small subunit (Green & Noller, 1997). Meanwhile, the peptidyl tRNA is in a similar hybrid situation with its acceptor end in the P site of the large subunit and its anticodon end in the A site of the small subunit (Kapp & Lorsch, 2004). This complex must then be translocated, so that the deacetylated tRNA is completely in the E site, the peptidyl tRNA completely in the P site, and the mRNA shifted by three nucleotides to place the next mRNA codon in the A site (Kapp & Lorsch, 2004). This process is mediated by eEF2, which hydrolyses GTP as it facilitates translocation (Wintermeyer et al., 2001). This cycle is then repeated until an mRNA stop codon is encountered and the termination process is initiated.

As an energy-expensive process, peptide elongation is tightly regulated under conditions that temporarily increase energy demand or reduce energy supply (Kapp & Lorsch, 2004; Kaul et al., 2011). Inhibition of peptide elongation therefore acts to preserve cellular energy, allowing it to be diverted to other vital cellular processes. Inhibition of protein synthesis at the elongation rather than initiation step confers advantage as it permits the retention of polysomes, thereby allowing for rapid resumption of peptide translation once cellular energy status is again favourable (Browne & Proud, 2002). Regulation of peptide elongation is primarily achieved via the eEF2 kinase (eEF2K), which upon activation phosphorylates and subsequently inhibits eEF2 at Thr56, subsequently inhibiting protein translation. The eEF2K is activated by stimuli induced during conditions of cellular energetic stress, including increased intracellular Ca^{2+} and cyclic AMP (cAMP) concentrations, increased AMP:ATP ratio and the associated activation of the 5’ AMP-activated protein kinase (AMPK), which result in eEF2K phosphorylation and subsequent activation (Browne & Proud, 2002). Conversely, the eEF2K is rendered inactive during periods of low energetic stress by signalling pathways positively regulating protein synthesis, including the mechanistic target of rapamycin (mTOR) pathway (discussed subsequently). The mTOR substrate p70S6K1 (70 kDa ribosomal protein subunit kinase 1) phosphorylates eEF2K on Ser366, while eEF2K phosphorylation at Ser359 is prevented in the presence of the mTOR inhibitor rapamycin, although the upstream kinase responsible for this phosphorylation is unclear (Browne & Proud, 2002). Phosphorylation of eEF2K at these residues leads to reduced eEF2 phosphorylation and subsequently eEF2 activation, allowing translation elongation to proceed (Browne & Proud, 2002).

Translation termination occurs when a stop codon (UAA, UAG, or UGA) is encountered in the ribosomal A site (Dever & Green, 2012). This process culminates in a completed polypeptide, which is released upon hydrolysis of the ester bond linking the polypeptide chain to the P site tRNA. This process is facilitated by two protein factors, eRF1 and eRF3 (eukaryotic release factors 1 and 3), which appear to collaborate in this process (Dever & Green, 2012). Upon recognition of the stop codon, an eRF1:eRF3:GTP complex binds to the ribosomal A-site, GTP hydrolysis occurs, and eRF3 is released. ABCE1/Rli1 binds to and facilitates eRF1 into an optimally-active configuration, subsequently promoting completed polypeptide release (Dever & Green, 2012).

In summary, protein synthesis is a highly complex process and is regulated primarily at the initiation and elongation steps (Kapp & Lorsch, 2004). The activity of key initiation and elongation factors is controlled by a number of upstream signalling cascades that relay stimuli such as mechanical loading and cellular energy status to influence rates of protein synthesis (Inoki et al., 2012). Two key pathways involved with the control of protein synthesis are the mTOR and AMPK pathways, which are further discussed in the following sections.

2.5.2 Mechanistic target of rapamycin (mTOR) signalling

The mechanistic target of rapamycin (mTOR) is a highly-conserved serine-threonine kinase that integrates both intracellular and extracellular signals to regulate cellular metabolism, growth, proliferation and survival (Inoki et al., 2012; Laplante & Sabatini, 2009; Ma & Blenis, 2009). The mTOR can be incorporated into two distinct functional complexes, the mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2) (Inoki et al., 2012). The mTORC1 (Figure 2.2) is a multi-protein complex comprised of mTOR, Raptor (regulatory associated protein of mTOR), mLST8 (mammalian lethal with SEC13 protein 8; also known as GβL [G-beta-L protein]), PRAS40 (proline-rich Akt substrate of 40 kDa) and Deptor (dishevelled, eg1-10, pleckstrin domain protein interacting with mTOR) (Kim et al., 2002; Kim et al., 2003; Peterson et al., 2009; Sancak et al., 2007), whereas the mTORC2 incorporates mTOR, Rictor (rapamycin-insensitive companion of mTOR), mLST8, mSIN1 (also known as mammalian stress-activated protein kinase interacting protein 1 [MAPKAP1]) and

Protor (also known as PRR5 [proline rich 5 (renal)]) (Pearce et al., 2007; Sarbassov et al., 2004; Woo et al., 2007; Yang et al., 2006).

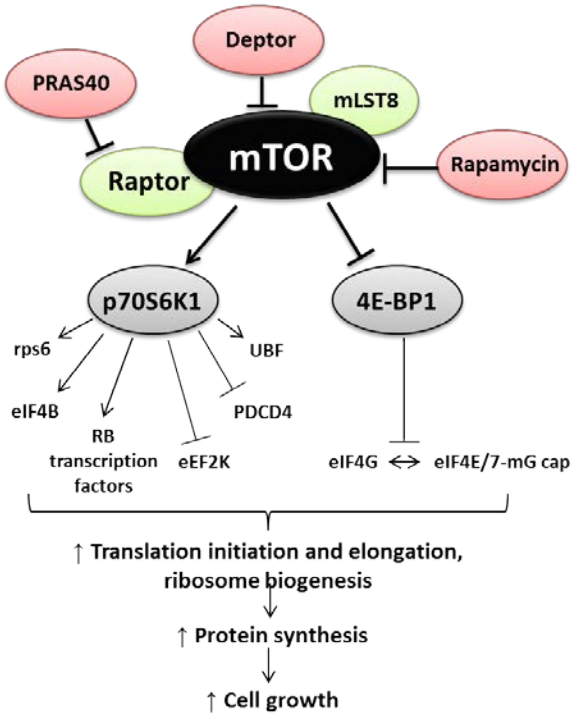

Figure 2.2 Summary of the mechanistic target of rapamycin complex 1 (mTORC1) components and its downstream protein synthesis-regulating signalling targets. Adapted from Goodman (2014).

The two mTOR complexes are functionally, as well as structurally, distinct. The mTORC1 is considered a master regulator of cell growth and metabolism and is directly regulated by cellular energy and nutrient status, while the mTORC2 is not (Inoki et al., 2012). Accordingly, mTORC1 promotes cell growth and proliferation by positively regulating anabolic processes such as protein synthesis, while inhibiting catabolic processes such as autophagy (Laplante & Sabatini, 2009). Conversely, the mTORC2 regulates processes distinct from mTORC1, including cell proliferation and survival (Goncharova et al., 2011), and actin cytoskeleton organisation (Jacinto et al., 2004). As the mTORC1 is the mTOR complex involved with the regulation of protein synthesis and cell growth, the following section of this review will solely focus on discussing the regulation of mTORC1.

2.5.2.1 Rheb/TSC2-mediated mTORC1 activation

The activity of mTORC1 is modulated by a number of upstream signals, including mechanical stress, growth factors, nutrients (including amino acids and glucose), and oxygen levels (Inoki et al., 2012; Laplante & Sabatini, 2009; Ma & Blenis, 2009). A key upstream player in the activation of mTORC1 is Rheb (Ras homolog enriched in brain), a small GTP-binding protein that possesses intrinsic GTPase activity (Aspuria & Tamanoi, 2004). In skeletal muscle, overexpression of Rheb activates mTORC1 signalling (Long et al., 2005b; Sato et al., 2009), stimulates protein synthesis, and induces muscle fiber hypertrophy (Goodman et al., 2011b; Goodman et al., 2010). When in its GTP-bound state, Rheb interacts with the mTOR catalytic domain, leading to increased mTORC1 activity (Long et al., 2005a). Interaction between active GTP-bound Rheb and mTORC1 appears to be an essential step in mTORC1 activation by all upstream signals (Laplante & Sabatini, 2009; Sancak et al., 2010). As Rheb functions as an important upstream regulator of mTORC1, it follows that factors modulating Rheb activity also regulate mTORC1. The GTP/GDP-binding status of Rheb is controlled by the tuberous sclerosis complex 2 (TSC2, also known as Tuberin). TSC2 exists in a complex with TSC1 (Hamartin) and functions as a GTPase activating protein (GAP) towards Rheb (Aspuria & Tamanoi, 2004; Inoki et al., 2003a). Early work implicated TSC1/2 activation with inhibiting the phosphorylation of p70S6K1 (p70 ribosomal protein S6 kinase 1) and 4E-BP1 (eukaryotic initiation factor 4E binding protein 1) (Goncharova et al., 2002; Manning et al., 2002; Tee et al., 2002), which was later shown to be mediated via inhibition of mTORC1 (Inoki et al., 2003a). The current model of TSC2/Rheb-mediated mTORC1 regulation dictates that under basal conditions, TSC2 stimulates the intrinsic GTPase activity of Rheb, subsequently converting active GTP-bound Rheb to inactive GDP-bound Rheb, and repressing mTORC1 signalling (Inoki et al., 2003a; Tee et al., 2002). Conversely, the GAP activity of TSC2 is inhibited by growth factors such as insulin in a phosphorylation-dependent manner, resulting in increased GTP-bound Rheb and subsequently increased mTORC1 signalling (Huang & Manning, 2008).

2.5.2.2 Growth factor-mediated mTORC1 activation

Growth factors, such as insulin and the insulin-like growth factor (IGF-1), have traditionally been implicated in the regulation of protein synthesis and skeletal muscle

hypertrophy (Goldberg, 1968). Mechanical stimuli are known to increase the expression of several IGF-1 isoforms, including a splice variant known as the mechano-growth factor (MGF), which purportedly acts in a paracrine manner (Hameed et al., 2003; Perrone et al., 1995). Early work demonstrated IGF-1 was sufficient to increase rates of protein synthesis (Gulve & Dice, 1989; Monier et al., 1983) and to induce skeletal muscle hypertrophy (Coleman et al., 1995). Importantly, IGF-1 was shown to act upstream of mTORC1 via the canonical phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway (Dardevet et al., 1996; Latres et al., 2005; Rommel et al., 2001). In this model of IGF-1-mediated mTORC1 activation (Figure 2.3), growth factor binding to the insulin receptor results in its auto-tyrosine phosphorylation and the subsequent recruitment of the insulin receptor substrate (IRS). Relocation and phosphorylation of IRS recruits PI3K to the cell membrane, which phosphorylates $PI4,5P_2$ (PIP2) to $PI4,5P_3$ (PIP3). PIP3 then recruits Akt (also known as protein kinase B [PKB]) and PDK-1 (phosphoinositol-dependent kinase-1) to the membrane, leading to Akt phosphorylation and subsequently activation (Dardevet et al., 1996; Latres et al., 2005; Rommel et al., 2001). Activated Akt then phosphorylates and inhibits the TSC1/2 complex (Inoki et al., 2002), blocking the TSC2-mediated inhibition of Rheb and subsequently leading to mTORC1 activation.

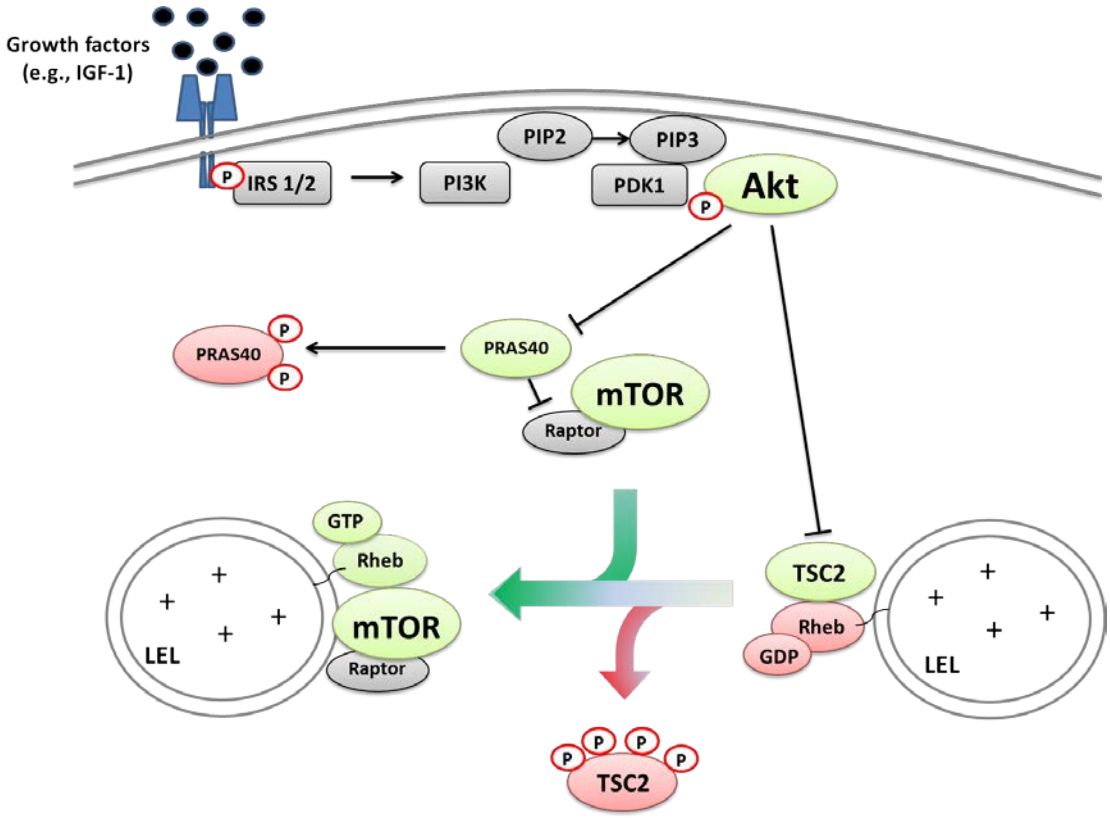

Figure 2.3 Regulation of the mechanistic target of rapamycin complex 1 (mTORC1) in response to growth factors. Growth factor (e.g., IGF-1) binding to the insulin receptor results in its auto-tyrosine phosphorylation and the subsequent recruitment of the insulin receptor substrate (IRS). Relocation and phosphorylation of IRS recruits PI3K (phosphatidylinositol 3-kinase) to the cell membrane, which phosphorylates $PI4,5P_2$ (PIP2) to $PI4,5P_3$ (PIP3). PIP3 then recruits Akt (also known as protein kinase B [PKB]) and PDK-1 (phosphoinositol-dependent kinase-1) to the membrane, leading to Akt phosphorylation and subsequently activation. Activated Akt then phosphorylates and inhibits both PRAS40 (proline-rich Akt substrate of 40 kDa) and TSC2 (tuberous sclerosis complex 2), blocking PRAS40-mediated inhibition of mTOR and preventing TSC2 from acting as a GAP towards Rheb (Ras homologue enriched in brain). These events allow mTORC1 to translocate to the late endosomal/lysosomal (LEL) membrane, where it encounters active GTP-bound Rheb, subsequently leading to mTORC1 activation. Adapted from Marcotte et al. (2015).

Adding support to the potential role of IGF-1 in mediating skeletal muscle hypertrophy were observations that treating myotubes with IGF-1 increased p70S6K1 phosphorylation and myotube size (Rommel et al., 2001), and that skeletal muscle loading increased IGF-1 mRNA expression (DeVol et al., 1990). It was therefore believed that increased mechanical loading of skeletal muscle by RE stimulated the autocrine release of IGF-1, which subsequently induced PI3K/Akt-dependent activation of the mTORC1 pathway, thereby increasing MPS and muscle growth (Philp et al., 2011b). However, several rodent models have demonstrated increased skeletal muscle mTORC1 signalling in response to mechanical stimuli occurring independently of IGF-1/Akt activation (Hamilton et al., 2010; Miyazaki et al., 2011; Philp et al., 2011b; Spangenburg et al., 2008). For example, in transgenic mice expressing a dominant-negative IGF-1 receptor in skeletal muscle that prevented receptor activation by insulin or IGF-1, load-induced muscle hypertrophy is identical to that of wild-type mice, although developmental growth was negatively affected, suggesting IGF-1/PI3K signalling may instead be required for developmental growth (Spangenburg et al., 2008). High-frequency electrical stimulation in mouse skeletal muscle, which is sufficient to increase mTORC1 signalling, is also not associated with markers of IGF-1 receptor activation, including increased tyrosine phosphorylation of either the IGF-1 receptor or PI3K p85 subunit (Hamilton et al., 2010). Adding further support to this hypothesis is the observation that inhibition of PI3K by wortmannin does not prevent the early (24 h) increase in mTORC1 signalling induced by synergist ablation in mice (Miyazaki et al., 2011). It should be noted, however, that IGF-1 may instead mediate skeletal muscle hypertrophy via the activation and differentiation of quiescent satellite cells, and the subsequent incorporation of new myonuclei into existing muscle fibres (Adams, 1998), although the necessity of satellite cell activation in muscle fibre hypertrophy remains contentious (Jackson et al., 2012; McCarthy & Esser, 2007a; McCarthy et al., 2011; O'Connor & Pavlath, 2007).

Additional work in human models has also shed light on the discordance between growth factors and the induction of MPS and skeletal muscle hypertrophy following RE. West and colleagues (West et al., 2010; West et al., 2009) employed a model in which RE involving divergent amounts of muscle mass (i.e., unilateral arm exercise performed with or without preceding lower body RE) was used to generate low- and high-systemic hormonal conditions, respectively. The high-hormone condition was successful in increasing systemic levels of total testosterone (5-fold), free

testosterone (3-fold), IGF-1 and growth hormone (both 10-fold) relative to the low-hormone condition. Regardless, these large divergences in systemic growth factor responses between conditions did not alter either mTORC1 signalling and rates of MPS (West et al., 2009), or muscle fibre hypertrophy and strength gains after 15 weeks of training (West et al., 2010). These data suggest there appears to be little role for the induction of systemic hormones and growth factors in promoting load-induced muscle fibre hypertrophy in human skeletal muscle.

2.5.2.3 Regulation of mTORC1 by amino acids

Amino acids are key regulators of the mTORC1 pathway (Inoki et al., 2012; Laplante & Sabatini, 2009; Ma & Blenis, 2009). The Rag (Ras-related GTP binding) proteins are small GTPases functioning as critical mediators of amino acid-dependent mTORC1 activation (Kim et al., 2008; Sancak et al., 2008). Four Rag proteins are expressed in mammalian skeletal muscle: RagA, RagB, RagC, and RagD, which form heterodimers consisting of RagA or RagB combined with RagC or RagD (Sancak et al., 2008). Amino acids promote the GTP-loading of heterodimers containing RagB or RagA, which then enables RagB or RagA to bind to the raptor component of mTORC1 (Sancak et al., 2008). The Rag proteins are anchored to the endosomal/lysosomal surface by the pentamic Ragulator complex, consisting of LAMTOR1 through to LAMTOR5 (late endosomal/lysosomal adaptor, MAPK and mTOR activator 1-5) (Bar-Peled et al., 2012; Sancak et al., 2010). The Ragulator complex not only tethers the Rags to the endosomal/lysosomal membrane, but also functions as their GEF (guanine nucleotide exchange factor) (Bar-Peled et al., 2012; Sancak et al., 2010). The GEF activity of Ragulator is specific to Rag heterodimers incorporating RagA or RagB (Bar-Peled et al., 2012). In response to high nutrient and energy levels, the V-ATPase (vacuolar H+-ATPase) stimulates the GEF activity of Ragulator, which catalyses the conversion of GDP-bound RagA/RagB to the GTP-bound form of RagA/RagB, subsequently increasing the affinity of Rags for mTORC1 (Bar-Peled et al., 2012; Efeyan et al., 2013; Zoncu et al., 2011). This stimulates the translocation of mTORC1 to the endosomal/lysosomal surface where the RagA/B heterodimer binds raptor, and mTORC1 is then activated after encountering its activator protein, Rheb (Bai et al., 2007; Sancak et al., 2010). Termination of this process is achieved by GATOR1 (GTPase-activating protein [GAP] activity towards Rags 1), which functions as a GAP

towards RagA/RagB (Bar-Peled et al., 2012), consequently promoting GDP-bound RagA/RagB and reducing the affinity of RagA/RagB for mTORC1. Under conditions of high amino acid availability, the GATOR2 complex moves the GATOR1 complex away from the Rag proteins, subsequently inhibiting the GAP activity of GATOR1 towards Rags (Bar-Peled et al., 2013). Amino acids therefore promote mTORC1 activation by promoting the endosomal translocation of mTORC1, which is directly mediated by the v-ATPase-Ragulator-Rag complex (Kim et al., 2008; Sancak et al., 2010; Sancak et al., 2008). The physiological advantage of a lysosomal-centred mechanism for mTORC1 activation may reflect the role of the lysosome in scavenging amino acids and other nutrients from cellular components via the catabolic process of autophagy (Efeyan et al., 2015). As lysosomal amino acid levels may at least partially reflect cellular amino acid abundance, the coupling of amino acid sensing with the regulation of mTORC1 and protein synthesis at the lysosome therefore confers a physiological advantage (Efeyan et al., 2015).

2.5.2.4 Regulation of mTORC1 signalling by mechanical loading

Accumulating evidence suggests mTORC1 activation induced by mechanical loading, like that stimulated by amino acids, is also associated with changes in the subcellular localisation of the mTORC1 and its associated regulatory proteins (Figure 2.4) (Jacobs et al., 2013; Sabatini et al., 1999; Sancak et al., 2010; Zhang et al., 2014). Early studies (Sabatini et al., 1999; Withers et al., 1997) established mTOR as a cytoplasmic protein that associates with intracellular membranes, which may translocate to specific subcellular compartments in response to certain stimuli (Sabatini et al., 1999). Indeed, the aforementioned Rag GTPase-mediated translocation of mTORC1 to the late endosomal/lysosomal membrane is an essential step in amino acid-mediated mTORC1 activation (Sancak et al., 2010). Like amino acids, mechanical loading has also been shown to modulate the subcellular localisation of mTORC1 and associated regulatory proteins in skeletal muscle to promote mTORC1 activation (Jacobs et al., 2013). For example, eccentric contractions in mouse skeletal muscle induce hyper-phosphorylation of TSC2 on RxRxxS*/T* residues, which almost completely abolishes any TSC2-lysosomal association, and simultaneously enhances the targeting of mTOR to the lysosome (Jacobs et al., 2013). The removal of TSC2 from the lysosomal membrane prevents its ability to act as a GAP towards Rheb (Aspuria & Tamanoi,

2004; Inoki et al., 2003a), consequently preventing TSC2-mediated Rheb inhibition and thus promoting mTORC1 activation. It therefore appears the translocation of mTORC1 and regulatory proteins in response to mechanical loading, as well as amino acid stimulation, represents an essential step in mTORC1 activation. Further work is required to examine changes mTORC1 translocation in response to different exercise and nutritional stimuli in human skeletal muscle to provide further insight into the modulation of mTORC1 activation by changes in its subcellular localisation.

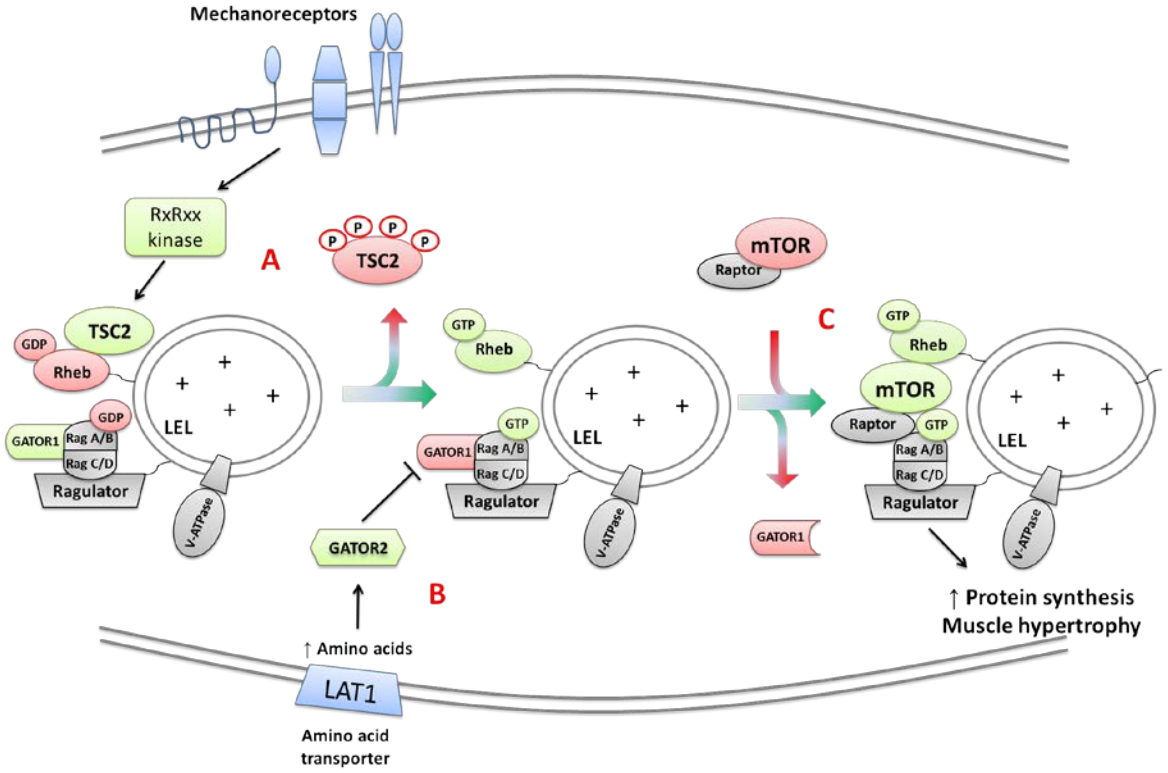

Figure 2.4 Regulation of mTORC1 by mechanical loading and amino acids. Mechanical loading stimulates mechanoreceptors in the cell membrane, which activate an unknown RxRxx kinase. This kinase then phosphorylates TSC2, subsequently removing TSC2 from the late endosomal/lysosomal (LEL) membrane and preventing it from acting as a GAP (GTPase-activating protein) towards Rheb (A). Simultaneously, increased cellular amino acid content activates GATOR2 (GAP activity towards Rags 2), which inactivates GATOR1 and prevents it from acting as a GAP towards Rag A/B heterodimers (B). Ragulator GTP subsequently loads the Rag A/B heterodimer, activating the complex. The active Rag complex then signals to Raptor, which induces translocation of mTORC1 towards GTP-bound Rheb at the LEL membrane (C). Adapted from Marcotte et al. (2015).

2.5.2.5 Regulation of downstream targets by mTORC1

The most well-characterised substrates downstream of mTORC1 are the p70 kDa ribosomal protein subunit kinase 1 (p70S6K1) and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) (Gingras et al., 1999; Yip et al., 2010). Both of these mTORC1 targets regulate protein synthesis via their influences on translation initiation and elongation, as well as ribosome biogenesis (Goodman, 2014), and are further discussed subsequently.

2.5.2.5.1 p70 kDa ribosomal protein subunit kinase 1 (p70S6K1)

The p70 ribosomal protein S6 kinase 1 (p70S6K1, also known as S6K1) is a highly-conserved Ser/Thr kinase belonging to the AGC family of protein kinases, and a key downstream target of the mTORC1 signalling cascade (Fenton & Gout, 2011; Ruvinsky & Meyuhas, 2006). p70S6K1 has an array of substrates and regulates diverse cellular processes including protein synthesis, mRNA processing, glucose homeostasis, cellular growth and survival. Like other members of the AGC protein family, p70S6K1 contains a hydrophobic motif that is C-terminal to the kinase domain, and to achieve full activity requires phosphorylation at multiple Ser/Thr residues through a series of phosphorylation events. Phosphorylation of p70S6K1 firstly occurs at multiple Ser residues (Ser411, Ser417, Ser421 and Ser424) located in the intrinsically disordered autoinhibitory domain (AID) at the C-terminus (Le et al., 2003) and the Ser371 residue in the turn motif linking the kinase domain and hydrophilic motif (Shin et al., 2011). To achieve full activation, p70S6K1 also requires phosphorylation at Thr389 in the hydrophobic motif, mediated by mTORC1 (Pearson et al., 1995), and phosphoinositide dependent kinase (PDK-1)-mediated phosphorylation at Thr229 in the activation loop (i.e., the 'T-loop') (Mora et al., 2004); however, the sequence of these events is unclear (Wang et al., 2013). The mTORC1 has long been implicated as an upstream regulator of p70S6K1 activity (Brown et al., 1995; Burnett et al., 1998; Isotani et al., 1999). Interaction between mTORC1 and p70S6K1 is facilitated by raptor, which binds directly to the TOR signalling motif on p70S6K1 (Nojima et al., 2003; Schalm & Blenis, 2002), subsequently permitting mTORC1-mediated phosphorylation at the Thr389 residue.

Once activated, p70S6K1 regulates protein synthesis by phosphorylating downstream targets implicated in translation initiation. The first identified p70S6K1

substrate was the ribosomal protein S6 (rps6), a component of the ribosomal 40S subunit believed to play a role in assembly of the translation initiation machinery (Banerjee et al., 1990; Price et al., 1990). The phosphorylation of rps6 by p70S6K1 occurs in a sequential fashion on multiple Ser residues (Ser236, Ser235, Ser240, Ser244 and Ser247) (Bandi et al., 1993; Krieg et al., 1988). However, the essential role of rps6 phosphorylation in regulating protein synthesis is unclear, given p70S6K1 and p70S6K2 knockout mice display normal translation of 5'-TOP (5'-tract of pyrimidine) mRNAs, which encode for translation factors and ribosomal proteins, despite the absence of rps6 phosphorylation (Pende et al., 2004; Ruvinsky et al., 2005). Despite this apparent paradox, p70S6K1 also positively regulates translation initiation via additional mechanisms, including the phosphorylation of the cap-binding complex component eIF4B at Ser422, and by phosphorylating and subsequently inhibiting PDCD4 (programmed cell death protein 4), a negative regulator of eIF4A (Dorrello et al., 2006). PDCD4 acts as a repressor of translation initiation by binding to the RNA helicase eIF4A and subsequently preventing its association with eIF4G, inhibiting translation initiation (Loh et al., 2009; Yang et al., 2003). The p70S6K1-mediated phosphorylation of PDCD4 stimulates its release from eIF4A and subsequent degradation by the ubiquitin-proteasome system (Dorrello et al., 2006; Zargar et al., 2011), facilitating eIF4A-eIF4G interaction and increased protein synthesis. In addition to regulating translation initiation, p70S6K1 also regulates the elongation step of protein translation via regulation of the eukaryotic elongation factor 2 kinase (eEF2K). Once activated, eEF2K negatively regulates translation elongation via phosphorylating the eukaryotic elongation factor 2 (eEF2) at the Thr56 residue (Wang et al., 2001). p70S6K1 inactivates the eEF2K by phosphorylating its Ser366 residue, relieving the eEF2k-mediated repression of translation elongation (Wang et al., 2001). More recent evidence also suggests the Ser366 phosphorylation of eEF2K may be a p70S6K1-independent, but mTORC1-dependent, event (Mieulet et al., 2007). In summary, p70S6K1 is a key downstream target of mTORC1 with a wide variety of substrates implicated in the regulation of translation initiation, elongation and ribosome biogenesis (see section 2.5.3). Phosphorylation of p70S6K1 at Thr389 is commonly used as a marker of mTORC1 activity, and correlations between early post-contraction increases in p70S6K1 phosphorylation and muscle growth following long-term training in both rodents (Baar & Esser, 1999) and humans (Mayhew et al., 2011; Terzis et al., 2008) highlight the potential importance of p70S6K1 in mediating load-induced muscle

growth. Despite these observations, the relationship between p70S6K1 activation and muscle growth is controversial, given others (Mitchell et al., 2012) have observed no clear relationships between p70S6K1 phosphorylation and long-term muscle hypertrophy.

2.5.2.5.2 Eukaryotic initiation factor 4E binding protein 1 (4E-BP1)

The activity of the eukaryotic initiation factor 4E binding protein 1 **(**4E-BP1), which acts as a repressor of translation initiation, is also controlled by mTORC1 (Gingras et al., 1999; Ma & Blenis, 2009). When hypo-phosphorylated, 4E-BP1 strongly interacts with eIF4E, consequently interfering with the binding of eIF4E to eIF4G, the subsequent formation of the eIF4F translation initiation complex, and recruitment of the 40S ribosomal subunit (Mahoney et al., 2009; Richter & Sonenberg, 2005). The phosphorylation of 4E-BP1 by mTORC1 facilitates the disassociation of 4E-BP1 from eIF4E, thus relieving the inhibitory effect of 4E-BP1 on eIF4E-dependent translation initiation (Gingras et al., 1999; Ma & Blenis, 2009; Richter & Sonenberg, 2005). Although a number of rapamycin-sensitive 4E-BP1 phosphorylation sites have been identified, mTORC1 preferentially phosphorylates 4E-BP1 on the Thr36 and Thr45 residues (Burnett et al., 1998; Gingras et al., 1999; Yang et al., 1999). The phosphorylation of 4E-BP1 by mTORC1 therefore plays a significant role in translation initiation and protein synthesis, although the necessity of mTORC1-mediated phosphorylation of 4E-BP1 for increases in protein synthesis and muscle fiber hypertrophy consequent to increased mechanical loading is not fully understood (Goodman, 2014).

In summary, activated mTORC1 regulates translation initiation via at least three mechanisms: 1) release of 4E-BP1 from eIF4E and 2) PDCD4 from eIF4A, enabling them to bind to eIF4G and form the active eIF4F complex (Gordon et al., 2013), and 3) phosphorylation of eIF4B which promotes assembly of eIF4A into the eIF4F complex (Park et al., 2013), with the latter two mechanisms mediated via p70S6K1.

2.5.3 AMPK (5' adenosine monophosphate-activated protein kinase) signalling

As a key physiological energy sensor, the 5' adenosine monophosphate-activated protein kinase (AMPK) is an important regulator of cellular energy homeostasis that coordinates various metabolic pathways to balance energy supply and demand, ultimately governing cellular and organ growth (Inoki et al., 2012). Mammalian AMPK is a highly-conserved heterotrimer comprised of a catalytic α- and regulatory β- and γ-subunits (Figure 2.5) (McGee & Hargreaves, 2010). Two α- (α1 and α2), two β- (β1 and β2) and three γ-subunits (γ1, γ2 and γ3) have been identified in mammalian cells, enabling 12 possible heterotrimeric combinations (Fogarty & Hardie, 2010). Three heterotrimeric complexes appear to predominate in skeletal muscle: α1/β2/γ1, α2/β2/γ1 and α2/β2/γ3, with the latter identified as the complex primarily activated by exercise in skeletal muscle (Wojtaszewski et al., 2005).

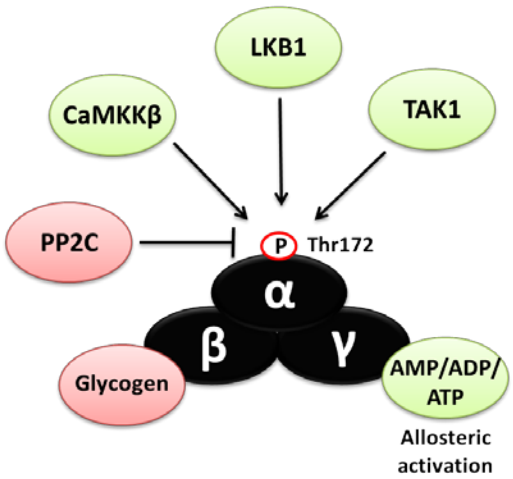

Figure 2.5 Heterotrimeric structure and upstream regulation of AMPK. Adapted from Richter and Ruderman (2009).

2.5.3.1 Upstream AMPK regulation

AMPK is activated in response to increased cellular AMP levels which arise during cellular metabolic stress (McGee & Hargreaves, 2010), an effect antagonised by high levels of cellular ATP (Corton et al., 1995). The binding of AMP to the CBS (cystathionine β-synthase) region of the γ-subunit allosterically activates the AMPK complex, causing a conformational change that prevents the pseudosubstrate domain from interacting with the α-subunit catalytic domain. This exposes the activation T-loop

of this domain to upstream kinases, which activate AMPK via phosphorylation on Thr172 (Steinberg & Kemp, 2009) and is essential for kinase activity (Scott et al., 2002; Stein et al., 2000). Additionally, the conformational change induced by AMP binding to the AMPK γ-subunit acts to inhibit the de-phosphorylation of Thr172 by phosphatases, particularly PP2C (protein phosphatase 2C) (Steinberg et al., 2006). These effects are entirely substrate-mediated, in that they are caused by AMP binding to AMPK and not to upstream kinases or phosphatases (Hardie & Sakamoto, 2006). Altogether, these AMP-mediated effects ensure AMPK is highly sensitive to alterations in the cellular AMP:ATP ratio (Hardie & Sakamoto, 2006; Inoki et al., 2012; McGee & Hargreaves, 2010). In many cell types, the major upstream AMPK kinase (AMPKK) is the LKB1 tumour suppressor, while there is evidence the Ca^{2+}/calmodulin-dependent kinase kinases (CaMKKs), particularly CAMKKβ, along with transforming growth factor β-activated kinase 1 (TAK1), also act as AMPKKs in some cell types (Hawley et al., 1995; Hong et al., 2003; Momcilovic et al., 2006). The net effect of AMP binding and the subsequent phosphorylation at Thr172 by upstream kinases profoundly increases AMPK activity by >100 fold (Jensen et al., 2009; Karagounis & Hawley, 2009). Commensurate with its role as a metabolic energy sensor, AMPK is activated by a host of factors that induce a state of cellular metabolic stress (i.e., increased AMP:ATP ratio), including muscular contraction, hypoxia, and/or hypoglycaemia (Steinberg & Kemp, 2009).

2.5.3.2 Downstream AMPK regulation

As a consequence of perturbations in cellular energy charge, AMPK acts to restore cellular energy balance by activating catabolic ATP-producing pathways while concomitantly inhibiting anabolic ATP-consuming pathways (McGee & Hargreaves, 2010) (Figure 2.6). Accordingly, AMPK is implicated in increasing lipid oxidation (Kaushik et al., 2001; Lee et al., 2006) via phosphorylation and inactivation of acetyl-CoA carboxylase (ACC) (Munday et al., 1988), stimulating insulin-independent glucose uptake in skeletal muscle by both increasing glucose transporter isoform 4 (GLUT4) gene expression via inhibition of histone deacetylase 5 (HDAC5) activity (McGee et al., 2008a), and increasing GLUT4 content at the sarcolemma via inhibition of TBC1D1 (TBC1 domain family member 1) and TBC1D4 (TBC1 domain family member 4, also known as AS160 [Akt substrate of 160 kDa]) (Merrill et al., 1997). Substrate delivery to

muscle is also regulated by AMPK via phosphorylation of endothelial nitric oxide (NO) synthase (eNOS), which alters vascular tone and subsequently increases NO production and tissue blood flow (Chen et al., 2009; McGee & Hargreaves, 2010).

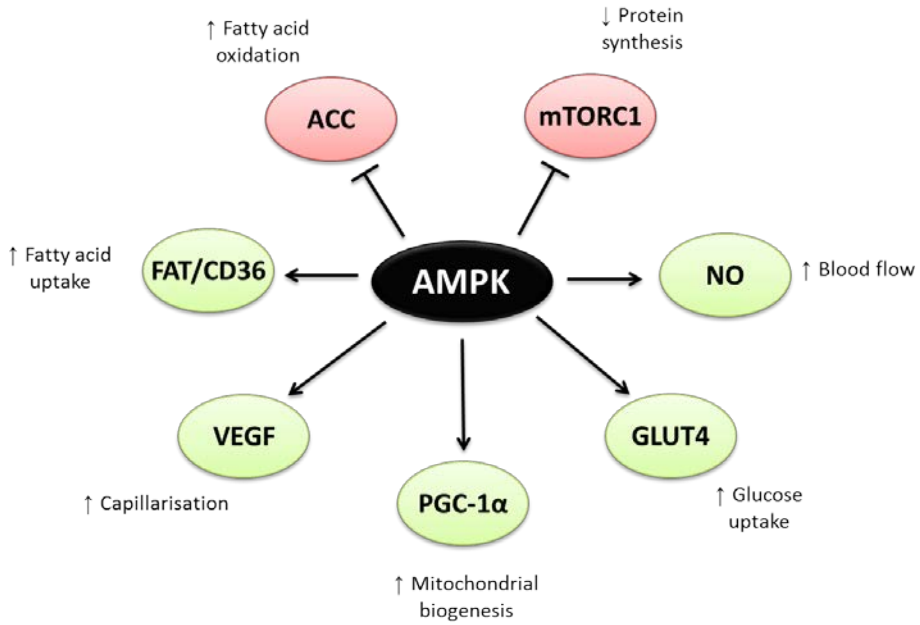

Figure 2.6 Summary of downstream targets of AMPK.

Along with enhancing the capacity for substrate supply into muscle and mitochondria, AMPK also controls the expression of a number of mitochondrial enzymes, such as those involved in lipid β-oxidation (β-hydroxyacyl-CoA dehydrogenase [β-HAD]) (Barnes & Zierath, 2005) and substrate flux into the tricarboxylic acid (TCA) cycle (pyruvate dehydrogenase kinase 4 [PDK4]) (Jorgensen et al., 2005), along with key enzymes in the TCA cycle (citrate synthase [CS]) (Jorgensen et al., 2007) and respiratory chain (COX [cytochrome *c* oxidase] subunits I and IV) (Garcia-Roves et al., 2008; Jorgensen et al., 2007), which collectively enhance substrate oxidative capacity. AMPK also modulates angiogenesis via regulation of the vascular endothelial growth factor (VEGF) gene, which is essential for increased muscle capillarisation and is associated with enhanced oxidative capacity (Zwetsloot et al., 2008), an effect mediated via the peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1α) (Leick et al., 2009; Olesen et al., 2010).

2.5.3.3 AMPK and mitochondrial biogenesis

AMPK also regulates a number of transcription factors and co-activators mediating both nuclear and mitochondrial gene expression, including the peroxisome-proliferator-activated receptor γ (PPARγ), PGC-1α, nuclear respiratory factors 1 and 2 (NRF-1/2), mitochondrial transcription factor A (Tfam) (Garcia-Roves et al., 2008; Lin et al., 2002) and the forkhead box O1 (Fox-O1) (Jorgensen et al., 2005). Principal among these downstream targets is PCG-1α, an inducible co-activator implicated in mitochondrial biogenesis and promoting shifts to a more oxidative and fatigue-resistant skeletal muscle phenotype (Lin et al., 2002; Olesen et al., 2010). PCG-1α partially exerts these effects via the co-activation of NRF 1 and 2, which are required for the coordinated expression of nuclear- and mitochondrial-encoded enzymes of the respiratory chain (Kelly & Scarpulla, 2004; Olesen et al., 2010). A single bout of endurance exercise induces rapid and sustained increases in PCG-1α mRNA and protein content in skeletal muscle (Mathai et al., 2008). Muscle-specific overexpression of PCG-1α in rodents induces a phenotype similar to endurance-trained muscle, including large increases in mitochondrial density, $\dot{V}O_{2max}$, shifts in fuel utilisation during submaximal exercise, and improved endurance performance (Calvo et al., 2008; Lin et al., 2002). Despite the marked changes in skeletal muscle phenotype induced by PCG-1α overexpression, knockout of PCG-1α does not prevent the exercise-induced increase in metabolic gene expression and mitochondrial biogenesis (Geng et al., 2010; Leick et al., 2008), indicating PCG-1α is not obligatory for these adaptations. PCG-1α is regulated by a number of post-translational modifications, including phosphorylation and deacetylation (Canto et al., 2009; Jager et al., 2007). AMPK regulates PCG-1α directly via phosphorylation and indirectly by phosphorylating and inhibiting the transcriptional repressor HDAC5 (histone deacetylase 5), subsequently relieving the HDAC5-mediated inhibition of MEF2 (myocyte enhancer factor 2), a known regulator of PCG-1α (McGee & Hargreaves, 2010). Another transcription factor implicated in mitochondrial biogenesis, the tumour suppressor protein p53, may also be regulated upstream by AMPK (Bartlett et al., 2014). Knockout of p53 in mouse skeletal muscle reduces PCG-1α expression, mitochondrial content and exercise capacity (Saleem et al., 2009), although not unlike PCG-1α (Perez-Schindler et al., 2013), p53 is also dispensable for exercise-induced mitochondrial biogenesis in mouse skeletal muscle (Saleem et al., 2009). Paradoxically, a recently-defined PGC-1α splice variant, termed

PGC-1α4, is induced by RE *in vivo* and may play a role in mediating skeletal muscle hypertrophy via up-regulation of IGF-1 and inhibition of myostatin (Ruas et al., 2012). Subsequent studies in humans have, however, questioned both the mode-specificity (Ydfors et al., 2013) and role of PGC-1α splice variants in skeletal muscle hypertrophy (Lundberg et al., 2014a).

2.5.3.4 AMPK-mediated regulation of protein synthesis

Given the role of AMPK in restoring cellular energy balance, AMPK is a key negative regulator of protein synthesis (Inoki et al., 2012; Kimball, 2006; Mounier et al., 2011), a process requiring a considerable amount of cellular energy. Multiple lines of evidence suggest AMPK negatively regulates mTORC1 and its downstream signalling targets, thereby inhibiting MPS and potentially muscle hypertrophy (Atherton et al., 2005; Bolster et al., 2002; Gwinn et al., 2008; Inoki et al., 2003a; Inoki et al., 2002; Inoki et al., 2003b). Supporting the role of AMPK in limiting muscle hypertrophy are observations that AMPK phosphorylation negatively correlates with muscle hypertrophy (Thomson et al., 2008) and is associated with attenuated muscle hypertrophy (Katta et al., 2012) in rodent functional overload models. Activation of AMPK has been shown to repress mTORC1 signalling via multiple mechanisms *in vitro*, including direct phosphorylation of the tuberous sclerosis complex 2 (TSC2) (Inoki et al., 2003b; McGee et al., 2008a) and the mTORC1-associated regulatory protein, raptor (Gwinn et al., 2008) (Figure 2.7). Activation of TSC2 by AMPK negatively regulates mTORC1 via inhibition of its upstream activator Rheb, subsequently blocking the downstream activation of regulators of protein translation (i.e., p70S6K1 and 4E-BP1) by mTORC1 (Bolster et al., 2002; Dreyer et al., 2006; Inoki et al., 2003b). The inhibitory effect of AMPK on Rheb is opposed by activated Akt, which phosphorylates and inactivates TSC2, subsequently alleviating its inhibition of mTORC1 (Hahn-Windgassen et al., 2005; Inoki et al., 2002). There is evidence the regulation of mTORC1 by AMPK may be AMPK isoform-specific. For example, it appears the AMPK α1 catalytic isoform is selectively responsible for limiting muscle hypertrophy via mTORC1 inhibition (McGee et al., 2008a; Mounier et al., 2011; Mounier et al., 2009), while AMPK α2 instead governs metabolic adaptations in skeletal muscle (Jorgensen et al., 2004; McGee et al., 2008a; Mounier et al., 2011). Evidence in rodent skeletal muscle suggests AMPK α1 is activated following chronic overload

(McGee et al., 2008a), and genetic knockout of this isoform results in greater hypertrophy (Mounier et al., 2009), supporting the isoform-specific role of AMPK in constraining muscle growth.

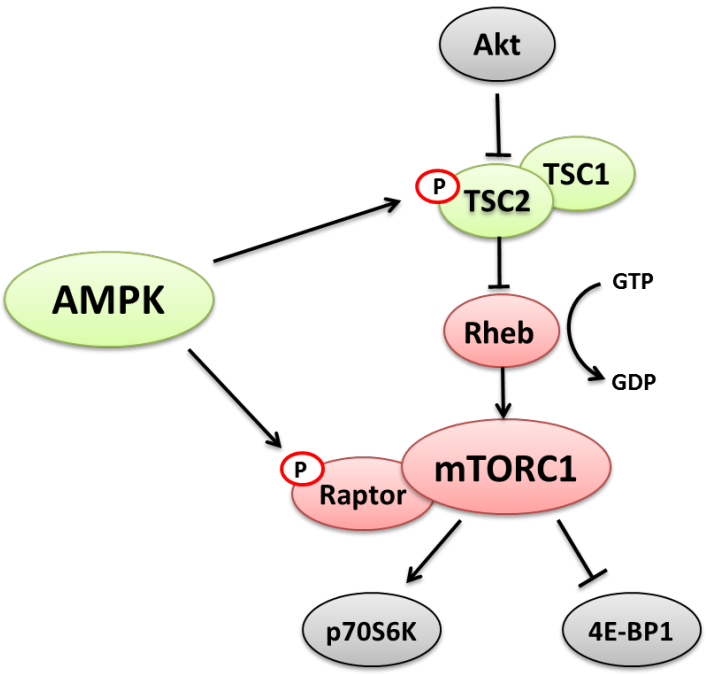

Figure 2.7 Regulation of the mTORC1 by AMPK. Under conditions of energy stress, activated AMPK phosphorylates TSC2, promoting its GAP activity towards the mTORC1 activator Rheb, subsequently converting active GTP-bound Rheb to inactive GDP-bound Rheb and inactivating mTORC1. Active AMPK also phosphorylates raptor, inhibiting mTORC1 activity. Adapted from Inoki et al. (2012).

2.5.1 Ribosome biogenesis-mediated regulation of protein synthesis

Rates of protein synthesis within the cell are determined by both translational efficiency (i.e., rates of protein synthesis per ribosome) and translational capacity (i.e., amount of translational machinery per unit of tissue, including ribosomes, tRNAs, and translational factors) (Chaillou et al., 2014). Ribosomes are supramolecular ribonucleoprotein complexes functioning at the heart of the translational machinery to convert mRNA transcripts into protein (Chaillou et al., 2014). Eukaryote ribosomes are composed of two ribosomal subunits. The small 40S subunit (comprising the 18S ribosomal RNA [rRNA] and 33 ribosomal proteins) is responsible for mRNA decoding

during translation initiation by aminoacyl-tRNA, whereas the large 60S subunit (comprising the 5S, 5.8S and 28S rRNAs and 47 ribosomal proteins), responsible for catalysing peptide bond formation during translation elongation via the peptidyltransferase reaction. Ribosomal content, which is indicative of cellular translational capacity, sets the upper limit of protein synthesis for the cell (Iadevaia et al., 2014). It is generally thought the stimulation of protein synthesis in the early hours following an anabolic signal (e.g., exercise or amino acid provision) is mediated by increased rates of translational efficiency, whereas repeated anabolic signals (i.e., weeks to months of exercise training) stimulate ribosome biogenesis and increases translational capacity (Chaillou et al., 2014). Ribosome biogenesis is there an important, yet overlooked, process closely linked to the regulation of skeletal muscle hypertrophy.

2.5.1.1 Molecular regulation of ribosome biogenesis

Ribosome biogenesis is a complex, well-orchestrated process involving transcription of the polycistrionic 45S rRNA precursor (45S pre-rRNA), processing of the 45S pre-rRNA into several smaller rRNAs (5.8S, 18S and 28S rRNAs), assembly of these rRNAs and other ribosomal proteins into ribosomal subunits (40S and 60S), and nuclear export of these ribosomal subunits into the cytoplasm (Chaillou et al., 2014; Thomson et al., 2013). The synthesis of the key components of the ribosomal subunits is achieved via the coordinated actions of three RNA polymerases (RNA Pol-I, -II, and –III). The RNA Pol-I is responsible for the transcription of the 45S pre-rRNA in the nucleolus, which is considered the rate-limiting step in ribosome biogenesis (Moss & Stefanovsky, 1995). The 45S pre-rRNA is subsequently cleaved into the 18S, 5.8S and 28S rRNAs, which undergo post-transcriptional modifications via interactions with small nuclear ribonucleoproteins and several protein processing factors. The RNA Pol-II is responsible for the transcription of ribosomal protein-encoding genes, whereas RNA Pol-III mediates the nucleoplasmic transcription of 5S rRNA and tRNAs. Ribosomal proteins comprising the small and large ribosomal subunits are subsequently translated in the cytoplasm, before being imported into the nucleolus where they are assembled with their respective ribosomal subunit. Once assembled, both the 40S and 60S ribosomal subunits are then exported into the cytoplasm where they form the mature 80S ribosome complex (Thomson et al., 2013).

As well as controlling translational efficiency, the mTORC1 is a key mediator of ribosome biogenesis by regulating transcription factors for genes encoding RNA Pol-I and -III (Iadevaia et al., 2014). The transcription of rDNA by RNA Pol-I requires the transcription factor SL-1 (selectivity factor-1), a component of which is TIF-1A (transcription initiation factor 1A; also known as RRN5), as well as other regulatory factors, including POLR1B (polymerase (RNA) 1 polypeptide B). Inhibition of mTORC1 by rapamycin inactivates TIF-1A, which impairs the transcription of the 45S pre-rRNA by RNA Pol-I (Mayer et al., 2004). Inhibition of mTORC1 also inactivates UBF (upstream binding factor) (Hannan et al., 2003), a transcription factor associated with SL-1, while the key mTORC1 substrate p70S6K1 promotes UBF activation and RNA Pol-I-mediated rDNA transcription (Hannan et al., 2003). The cyclins (including cyclin-D1) and cyclin-dependent kinases (CDKs) can also regulate UBF via phosphorylation on Ser388 and Ser484, which are required for UBF activity (Voit & Grummt, 2001; Voit et al., 1999). In addition to regulation of RNA Pol-1, mTORC1 also associates with a number of RNA Pol-III genes that synthesise both 5S rRNA and tRNA (Kantidakis et al., 2010).

2.5.1.2 Ribosome biogenesis and skeletal muscle hypertrophy

Evidence gleaned from rodents (Adams et al., 2002; Chaillou et al., 2012; Chaillou et al., 2013; Goodman et al., 2011a; Miyazaki et al., 2011; Nakada et al., 2016; von Walden et al., 2012) suggests skeletal muscle hypertrophy following chronic overload is accompanied by ribosome biogenesis, as evidence by increased RNA content, 80-85% of which is comprised of rRNA (Chaillou et al., 2014). Moreover, attenuated skeletal muscle hypertrophy in ageing rodent muscle following chronic overload (Kirby et al., 2015) and in a model of chronic inflammatory bowel disease (Figueiredo et al., 2016a) is associated with attenuated ribosome biogenesis. Although limited data exists in humans (Figueiredo et al., 2015; Nader et al., 2014; Stec et al., 2015), total RNA content and several markers of ribosome biogenesis, including UBF mRNA, phosphorylated UBF (Ser388), the pre-45S rRNA and the mature 28S, 18S and 5.8S transcripts, are upregulated in human skeletal muscle at rest following 8 weeks of RT (Figueiredo et al., 2015). Single bouts of RE also increase TIF-1A phosphorylation and levels of cyclin D1 protein in human skeletal muscle (Figueiredo et al., 2015), mRNA levels of the 45S rRNA precursor (Figueiredo et al., 2015; Nader et al., 2014;

Stec et al., 2015), and the mature 5.8S, 18S and 28S rRNAs (Figueiredo et al., 2015), as well as other regulators of ribosome biogenesis, including c-Myc (Nader et al., 2014). A blunting of responses related to ribosome biogenesis in skeletal muscle has been demonstrated in the 48 h after the application of cold-water immersion following a single bout of RE in humans (Figueiredo et al., 2016b), an approach shown to attenuate adaptations to long-term RT (Roberts et al., 2015). Ribosome biogenesis therefore appears to be an important, yet overlooked, process occurring during RT-induced skeletal muscle hypertrophy (Chaillou et al., 2014; Figueiredo et al., 2015). Further work is required to determine the necessity of ribosome biogenesis in skeletal muscle hypertrophy and its regulation both in human skeletal muscle following different types of exercise training, and in situations where muscle hypertrophy might be compromised (e.g., during concurrent training).

2.5.2 microRNA-mediated regulation of protein synthesis

Additional molecular mechanisms with the potential to regulate skeletal muscle adaptations to exercise have recently emerged, including altered microRNA (miRNA) expression (Zacharewicz et al., 2013). miRNAs are small (~20-30 nucleotides in length), non-coding ribonucleic acid (RNA) species highly expressed in skeletal muscle (Zacharewicz et al., 2013). The primary function of miRNAs is to decrease protein levels either by repressing protein translation or promoting the degradation of target mRNAs, of which the latter accounts for the majority of miRNA activity (Guo et al., 2010). Given their purported role in post-transcriptional regulation, miRNAs have emerged as a potential regulator of exercise-induced adaptations in skeletal muscle (Zacharewicz et al., 2013). The expression of various miRNA species in human skeletal muscle is altered following both single bouts of exercise (Drummond et al., 2008c; Russell et al., 2013; Zacharewicz et al., 2014) and after short-term exercise training (Davidsen et al., 2011; Russell et al., 2013). miRNAs have been implicated in the regulation of the IGF-1/Akt, Fox-O1, and myogenesis pathways, all known to play a role in exercise-induced adaptations in skeletal muscle (Hitachi & Tsuchida, 2013), although the direct impact of miRNAs on these pathways in skeletal muscle consequent to RE is yet to be experimentally validated. The myomiRs miR-1 and miR-133a can target members of the IGF-1/Akt pathway *in vitro*, including both IGF-1 and the IGF-1 receptor (Elia et al., 2009) as well as HSP70 (Kukreti et al., 2013). This suggests they

may play a role in regulating muscle hypertrophy, although this requires experimental validation *in vivo*. Expression of both miR-1 and miR-133a is reduced during functional overload in mice (McCarthy & Esser, 2007b), and miR-1 expression is reduced 1 h after a single bout of RE with amino acid ingestion (Drummond et al., 2008c), whereas a single bout of endurance exercise increases miR-1 expression (Russell et al., 2013). Reduced post-exercise miR-1/miR-133a expression may therefore alleviate repression of IGF-1/Akt signalling by these miRNAs. Other miRNA species, including miR-378 and miR-486, have been purported to regulate molecular pathways related to myogenesis, protein synthesis and degradation. miR-378 has been implicated in myogenesis by negatively regulating MyoR, a negative upstream regulator of the transcription factor MyoD (myogenic differentiation 1) (Gagan et al., 2011). Low responders to RE-induced lean mass gain after 12 weeks of RE training show reduced basal miR-378 expression, whereas miR-378 expression is unchanged in those classified as high responders (Davidsen et al., 2011). The change in miR-378 expression has also been correlated ($r^2 = 0.52$) with lean mass gain after RE training (Davidsen et al., 2011), suggesting maintenance of miR-378 expression may be necessary for promoting RE-induced muscle growth. miR-486 has been linked to the regulation of skeletal muscle mass by targeting components of the Akt pathway, including PTEN (phosphatase and tensin homologue), an upstream inhibitor of Akt, and by negatively regulating the transcription factor Fox-O1 (Xu et al., 2012), a mediator of ubiquitin ligase expression and subsequently protein degradation (Sandri et al., 2004). Increased post-exercise miR-486 expression, when repeated over time, may therefore be favourable for promoting anabolism in skeletal muscle by alleviating PTEN-mediated repression of Akt, and suppressing Fox-O1-mediated ubiquitin ligase expression. Conversely, reduced miR-486 expression may suggest reduced inhibition of PTEN and Fox-O1, and subsequently reduced Akt signalling and increased ubiquitin ligase expression.

Taken together, there is evidence to suggest altered post-exercise miRNA expression may play a role in mediating exercise-induced adaptations in skeletal muscle. Further work is required, however, to determine the functional roles of these miRNAs in human skeletal muscle following exercise, and their subsequent effects on skeletal muscle adaptations after prolonged training. Given that miRNAs may play a role in regulating molecular signalling pathways related to muscle hypertrophy and/or myogenesis (Hitachi & Tsuchida, 2013; Zacharewicz et al., 2013), together with observations of divergent miRNA expression between single bouts of endurance

(Russell et al., 2013) and RE (Drummond et al., 2008c), suggests miRNA expression is worthy of examination following concurrent exercise. Whether divergent miRNA expression in skeletal muscle following concurrent exercise compared with single-mode RE might play a role in adaptation to long-term concurrent training is currently unclear, and has not been examined to date.

2.6 Molecular regulation of skeletal muscle protein breakdown

A decrease in skeletal muscle fibre CSA is termed muscular atrophy. Like muscle fibre hypertrophy, muscular atrophy is determined by the net balance of MPS, and occurs when rates of MPB are persistently above those of synthesis (Sandri, 2013). Muscle fibre atrophy is therefore governed by signalling cascades mediating increased rates of MPB and/or the inhibition of MPS. Two major protein degradation systems are known to operate in eukaryotic cells: the ubiquitin-proteasome and autophagy-lysosomal systems (Sandri, 2013). The ubiquitin-proteasomal system is generally responsible for the degradation of short-lived proteins, whereas the autophagy-lysosomal system mediates breakdown of longer-lived cellular proteins (Fanzani et al., 2012). Although increased rates of MPB may be seen as counteractive to muscle anabolism and subsequently adaptation, ubiquitin-proteasome and/or autophagy-lysosomal activity may be necessary to facilitate exercise-induced skeletal muscle remodelling by removing damaged proteins and/or providing amino acid substrates for incorporation into newly synthesised proteins (Sanchez et al., 2014a; Vainshtein & Hood, 2015).

2.6.1 Ubiquitin-proteasomal system

The ubiquitin-proteasomal system involves the tagging of substrate proteins with ubiquitin, an abundant 8-kDa protein, which subsequently targets the substrate protein for degradation by the 26S proteasome (Fanzani et al., 2012). The process of protein ubiquitination is characterised by a series of reactions. Ubiquitin is firstly activated by an ubiquitin-activating enzyme (E1), which subsequently transfers activated ubiquitin to the active site of an ubiquitin-conjugated enzyme (E2). Finally, E2 binds to an E3 ligase, which is responsible for recognising and ubiquitinating the

target substrate. The process of protein ubiquitination in skeletal muscle is mediated by two muscle-specific E3 ubiquitin ligases; MuRF-1 (muscle RING finger-1) and MaFbx/Atrogin-1 (muscle atrophy F-box) (Bodine et al., 2001a). Murine knockout of MuRF-1 or MaFbx confers partial protection against muscle wasting under various atrophic conditions, including denervation, dexamethasone treatment, and fasting (Baehr et al., 2011; Bodine et al., 2001a; Gomes et al., 2012). Known MuRF-1 targets include several muscle structural proteins, such as the myosin heavy chains (MyHCs), the most abundant skeletal muscle proteins (Schulze et al., 2005). Other myofibrillar proteins, including actin (Polge et al., 2011), titin (Centner et al., 2001; McElhinny et al., 2002), troponin 1 (Kedar et al., 2004), myosin binding protein C, and myosin light-chains 1 and 2 (Cohen et al., 2009), are also specifically ubiquitinated by MuRF-1. Whereas MuRF1 possesses a wide variety of known substrates, the only confirmed targets of Atrogin-1 are MyoD (Tintignac et al., 2005), a transcription factor mainly involved with the myogenic program, and the translation initiation factor eIF3F (Lagirand-Cantaloube et al., 2008). Moreover, the role of Atrogin-1 in protein degradation remains unclear, as Atrogin-1 mRNA levels do not correlate with rates of protein degradation both *in vivo* (Krawiec et al., 2005) and *in vitro* (Dehoux et al., 2007). The expression of MuRF-1 and Atrogin-1 are positively controlled by the Fox-O subfamily of transcription factors, including Fox-O1 and Fox-O3a (Kamei et al., 2004; Sandri et al., 2004). The Fox-O proteins have broad cellular roles including tumour suppression, protein degradation, and development in several tissues, and are regulated by phosphorylation-dependent nuclear/cytoplasmic shuttling (Calnan & Brunet, 2008; Sanchez et al., 2012a). Phosphorylation of the Fox-O proteins, mediated by Akt, stimulates their exclusion from the nucleus to the cytoplasm by 14-3-3 protein binding (Brunet et al., 1999). Conversely, de-phosphorylated Fox-O transcription factors translocate to the nucleus where they can act as transcription factors and promote the expression of MuRF-1 and Atrogin-1 (Sandri et al., 2004). Indeed, increased MuRF-1 and Atrogin-1 expression following *in vitro* treatment of myotubes with dexamethasone was shown to be antagonised by the simultaneous treatment of IGF-1 acting through the PI-3K/Akt pathway (Sandri et al., 2004; Stitt et al., 2004). AMPK also positively regulates Fox-O3a via phosphorylation at Ser413/588, residues known to activate Fox-O3a and protein degradation (Greer et al., 2007). Thus, AMPK regulates anabolism not only by inhibiting protein synthesis, but also by mediating protein degradation via both the ubiquitin-proteasome and autophagy-lysosomal systems (discussed in section 2.6.2).

2.6.2 Autophagy-lysosomal system

Autophagy is a constitutively-active and evolutionarily-conserved process for the bulk degradation and recycling of proteins and entire organelles via the lysosomal machinery (Klionsky, 2007). The induction of autophagy firstly involves the formation of double-membrane vacuoles known as autophagosomes, which package and deliver cytoplasmic material to the lysosomes for subsequent degradation (Xie & Klionsky, 2007). After autophagosome formation, the isolation membrane (or phagophore) elongates and engulfs a portion of the cytoplasm or specific cargo, forming a double membrane-bound autophagosome (Eskelinen & Saftig, 2009). The mature autophagosome then fuses with lysosomal vesicles, forming an autolysosome. The fusion of the outer autophagosomal membrane with the lysosomal membrane permits the degradation of the inner autophagosomal membrane and its cytoplasmic contents by lysosomal hydrolases (Eskelinen & Saftig, 2009). The degradation products are then transported back to the cytoplasm, where they can be re-used for biosynthesis or energy production (Eskelinen & Saftig, 2009). In skeletal muscle, autophagy is activated during a plethora of catabolic conditions, including disuse (Brocca et al., 2012), denervation (Zhao et al., 2007), fasting (Mammucari et al., 2007), and ageing (Wohlgemuth et al., 2010). Autophagy is also induced by exercise in human skeletal muscle (Jamart et al., 2012a; Jamart et al., 2012b), which may function to remove proteins and organelles damaged by exercise itself, or to provide energy for sustained contractile activity (Sandri, 2013). Autophagy requires Atg (autophagy-specific gene) proteins, which are necessary for the formation of autophagosomes (Codogno, 2004). Accumulating evidence suggests autophagy is under the antagonistic control of mTORC1 and AMPK, which both regulate ULK1 (Unc-51-like kinase 1), a serine/threonine protein kinase with a key role autophagy induction (Inoki et al., 2012; Kim et al., 2011). Under nutrient-rich conditions, mTORC1 interacts with and phosphorylates ULK1 on Ser757 (Kim et al., 2011). This phosphorylation of ULK1 by mTORC1 blunts ULK activity and/or prevents its association with other co-factors including Atg13 and FIP200, which are necessary processes for coordinating the autophagy response (Ganley et al., 2009). During cellular energy stress, activated AMPK inhibits mTORC1 via phosphorylation of TSC2 and raptor (Gwinn et al., 2008; Inoki et al., 2003b), possibly disrupting the mTORC1-ULK1 interaction and preventing the inhibitory phosphorylation of ULK1 by

mTORC1. This allows AMPK-mediated phosphorylation of ULK1 on multiple residues, which may also increase ULK activity (Sanchez et al., 2012a). In addition to regulating ULK1, AMPK-mediated activation of Fox-O3a stimulates increased expression of factors necessary for autophagosome formation, including Beclin, LC3-II and Gabarapl1 (Sanchez et al., 2012b).

2.7 Molecular basis for the specificity of training adaptation

In recent years, insight into the molecular factors mediating skeletal muscle adaptations to divergent exercise modes has emerged (Atherton et al., 2005; Bodine et al., 2001b; Pilegaard et al., 2003; Stitt et al., 2004). Exercise-induced adaptations in skeletal muscle are considered the cumulative result of early molecular signalling responses and subsequent gene expression stimulated by repeated exercise bouts, leading to the accumulation of specific proteins over time and, subsequently, an altered muscle phenotype (Egan et al., 2013; Fluck & Hoppeler, 2003; Perry et al., 2010). It has been suggested resistance and endurance exercise stimulate almost distinct activation of specific molecular signalling pathways and gene networks mediating the mode-specific adaptations to chronic exercise training (Atherton et al., 2005; Coffey & Hawley, 2007; Hawley, 2009). As discussed in earlier sections of this review, muscle fibre hypertrophy induced by RE is generally considered to be mediated by the anabolic mTORC1 signalling cascade (Bodine et al., 2001b). The necessity of mTORC1 for load-induced skeletal muscle growth is supported by evidence that rapamycin (a selective mTORC1 inhibitor) administration prevents both muscle hypertrophy *in vivo* (Bodine et al., 2001b) and the increase in MPS following a single bout of RE in humans (Drummond et al., 2009). Conversely, adaptations induced primarily by endurance exercise, including mitochondrial biogenesis (Baar et al., 2002; Pilegaard et al., 2003; Wu et al., 2002), improved substrate utilisation (Holloszy & Coyle, 1984) and increased capillary density (Saltin & Gollnick, 1983), are purportedly mediated by the AMPK and Ca^{2+}/calmodulin-dependent kinase II (CaMKII) pathways, among others, which converge on the transcriptional co-activator PGC-1α (Baar et al., 2002; Pilegaard et al., 2003; Wu et al., 2002).

2.7.1 The AMPK/Akt "master switch" hypothesis

Early insight into the molecular basis for the specificity of training adaptation came from the work of Atherton and colleagues (Atherton et al., 2005). These workers employed a model in which isolated rat muscle was electrically stimulated at either high (6 x 10 3-s repetitions at 100 Hz for 20 min) or low (3 h at 10 Hz) frequencies to mimic resistance or endurance exercise, respectively. They observed selective activation of the anabolic Akt/mTOR signalling cascade by resistance-like stimulation, with little effect on the AMPK/PGC-1α pathway, while endurance-like stimulation caused increased AMPK activation and PGC-1α protein levels (Atherton et al., 2005). Moreover, endurance-like stimulation inhibited Akt/mTOR and its downstream targets. Therefore, these authors postulated selective activation of either Akt/mTOR or AMPK/PGC-1α could explain the divergent adaptations associated with resistance and endurance training, respectively, in a paradigm termed the "AMPK/Akt master switch" hypothesis (Atherton et al., 2005). While this is an attractive regulatory model, the existence of such clear divergence in humans has to date proven elusive (Camera et al., 2010; Coffey et al., 2006a; Coffey et al., 2006b; Vissing et al., 2011; Wilkinson et al., 2008). This is perhaps not surprising, given the *ex-vivo* electrical stimulation rodent model employed by Atherton and colleagues (2005) arguably represents few similarities to contracting human skeletal muscle during exercise. Indeed, several observations question the simplistic notion of an AMPK/Akt "master switch" mediating training adaptation specificity in human skeletal muscle, while highlighting the complexity of exercise-induced molecular responses. For example, a number of human studies have not observed notable differences in either mTORC1 or AMPK signalling in the early recovery period following endurance and RE performed separately (Camera et al., 2010; Coffey et al., 2006b; Vissing et al., 2011; Wilkinson et al., 2008). Several studies have shown increased mTORC1 activity following endurance exercise in human skeletal muscle (Benziane et al., 2008; Mascher et al., 2007; Mascher et al., 2011), which may reflect the putative role for mTORC1 in regulating oxidative metabolism (Bentzinger et al., 2008; Cunningham et al., 2007; Schieke et al., 2006), while RE can increase AMPK phosphorylation (Coffey et al., 2009a; Coffey et al., 2006b; Dreyer et al., 2006; Koopman et al., 2006; Vissing et al., 2011; Wilkinson et al., 2008). Regardless of the exercise modality, other independent factors can modulate the molecular responses to exercise, including training status (Benziane et al., 2008; Coffey et al., 2006a; Coffey et

al., 2006b; McConell et al., 2005), age (Drummond et al., 2008a; Fry et al., 2011), genetic factors (Raue et al., 2012; Timmons et al., 2010) and nutrient availability (Churchley et al., 2007; Creer et al., 2005; Deldicque et al., 2005; Yeo et al., 2010). The molecular factors mediating the specificity of training adaptation are clearly more complex than dictated by a simplistic "master switch" model (Atherton et al., 2005), and further work is required to more completely define the mechanisms responsible.

2.7.2 Molecular basis for the specificity of training adaptation in human skeletal muscle

2.7.2.1 Exercise mode-specific divergences in early signalling responses following single bouts of exercise

Several studies have investigated the signalling mechanisms underlying the specificity training adaptation in human skeletal muscle (Camera et al., 2010; Moller et al., 2013; Vissing et al., 2011; Wilkinson et al., 2008). Camera and colleagues (2010) determined the early time course of post-exercise signalling responses after prolonged cycling (60 min at 70% $\dot{V}O_{2peak}$) compared to high-intensity RE (8 x 5 leg-extension repetitions at 80% 1-RM [one-repetition maximum]). Despite clear divergence in the duration and intensity of the contractile stimulus, no significant differences were noted in early translational signalling responses (i.e., Akt/mTOR/p70S6K1 phosphorylation) in the 60-min post-exercise period, while only endurance exercise promoted the activation of signalling proteins involved in glucose uptake and glycogen synthesis (Camera et al., 2010). It was therefore concluded resistance and endurance exercise were equally capable of stimulating translation-initiation signalling, including mTOR and p70S6K1 phosphorylation, at least during early recovery. Divergent AMPK responses were however observed between conditions, with AMPK Thr172 phosphorylation increased above baseline at all time-points after cycling exercise, whereas RE did not increase AMPK phosphorylation during the 1 h post-exercise period (Camera et al., 2010).

To limit the potentially confounding effects of training status on molecular responses to exercise (see section 2.8.2.2), Vissing and colleagues (2011) compared signalling responses to single bouts of resistance and endurance exercise in subjects accustomed to either exercise mode after 10 weeks of prior training. The major finding of that study was components of the mTORC1 cascade (i.e., mTORC1, p70S6K1 and

AS160 [Akt substrate of 160 kDa]) responded preferentially to RE and not to prolonged, low-intensity endurance exercise, whereas AMPK was not differentially activated following either exercise mode (Vissing et al., 2011). Activation of mTORC1 and its downstream targets were not associated with the concomitant activation of Akt, supporting previous observations of the discordance between Akt activation and mTORC1 signalling (Goodman et al., 2010; Hornberger et al., 2006). It therefore appears that in training-accustomed individuals, mTORC1 signalling becomes more exercise modality-specific and is preferentially activated following resistance, but not endurance exercise.

A subsequent study (Moller et al., 2013) expanded on the work of Vissing et al. (2011) and investigated additional molecular targets that may be differentially regulated by resistance and endurance exercise. In particular, signalling cascades activated by inflammation and cellular stress (i.e., NF-κB [nuclear factor kappa-light-chain-enhancer of activated B cells] and the mitogen-activated protein kinases [MAPK] ERK [extracellular signal-regulated kinase] 1/2 and p38) and myokines (i.e., interleukin [IL] 1β, IL6, IL8 and TNFα [tumor necrosis factor alpha]) were investigated. The NF-κB, ERK 1/2 and p38 have been suggested to link inflammatory responses to the regulation of protein synthesis via the TSC1/2 complex. ERK 1/2 and p38 purportedly impact upon protein synthesis by phosphorylating TSC2 (Ma et al., 2005) and raptor (Wu et al., 2011), respectively. Additionally, the IKKβ [inhibitor of nuclear factor kappa-B kinase subunit beta] -mediated phosphorylation of TSC1 disrupts the TSC1/TSC2 complex, leading to mTORC1 activation (Lee et al., 2007; Lee et al., 2008). Interestingly, IKKβ was found to be preferentially activated following RE in a manner mirroring the phosphorylation of mTORC1. However, this was not associated with concomitant phosphorylation of TSC1 at either Ser511 or Ser487, the latter of which may have been due to technical limitations. The link between IKKβ and mTORC1 activation was therefore unclear; however, the authors could not completely rule out the IKKβ-mediated phosphorylation of TSC1 at Ser487 as a potential mechanism. Moreover, neither p38 nor ERK 1/2 were activated in response to resistance or endurance exercise, which was in agreement with previous evidence from highly-trained strength and endurance athletes (Coffey et al., 2006b). It was therefore suggested the activation of IKKβ, which occurred exclusively following RE in training-accustomed individuals, might be an upstream signal mediating the specificity of training adaptation via mTORC1 activation.

2.7.2.2 Exercise mode-specific divergences in synthesis of specific myocellular protein pools

In addition to potential divergences in early post-exercise signalling responses, it is conceivable that divergent exercise modalities stimulate distinct increases in the synthesis of specific myocellular protein pools, which accumulate over time to generate the chronic mode-specific phenotypes. Using a unilateral training model, Wilkinson and colleagues (2008) showed RE performed in the untrained state stimulated almost equivalent increases in myofibrillar and mitochondrial protein synthesis, whereas this balance was shifted towards synthesising only myofibrillar proteins after 10 weeks of RE training. On the other hand, endurance exercise increased mitochondrial protein synthesis equally both before and after training, with no detectable increases in myofibrillar protein synthesis (Wilkinson et al., 2008). However, the differential stimulation of fractional protein synthesis pools was not associated with clear divergences in activation of various components of the translation-initiation machinery. The only notable between-mode differences seen were that RE resulted in a more prolonged p70S6K1 phosphorylation response and a later phosphorylation of rps6 and eIF4E (Wilkinson et al., 2008). Thus, while divergent exercise modes clearly have the capacity to differentially stimulate specific myocellular protein pools, which become more refined with training, these changes are not necessarily associated with detectable differences in phosphorylation of the translation initiation machinery. Given rates of protein synthesis are increased regardless of the exercise modality; it is perhaps conceivable the translational machinery might also be similarly activated. However, the upstream signals responsible for firstly decoding the mode-specific exercise signal and then mediating the specific increases in fractional rates of protein synthesis remain to be determined.

2.7.2.3 Critical stimuli mediating the specificity of training adaptation

As previously discussed (see section 2.2), skeletal muscle adaptations to exercise are generally accepted to be specific to the modality of exercise performed, which is in turn related to the intensity and duration of the contractile stimulus (Hawley, 2009). For example, traditional continuous-style endurance exercise involves low-intensity, long-duration contractile activity, whereas typical RE is characterised by relatively high-intensity, short-duration muscular contractions. However, endurance-like

molecular responses and adaptations are induced in human skeletal muscle by single bouts of repeated-sprints (Serpiello et al., 2012) and low-volume HIT (Gibala et al., 2006; Gibala et al., 2009), both of which are characterised by relatively high-intensity, short-duration contractions resembling those of RE. These observations raise questions about the upstream signals dictating the divergent adaptations induced in skeletal muscle by resistance and endurance exercise. The critical factors explaining this apparent paradox appear to be divergences in both the mechanical loading and metabolic stress experienced by skeletal muscle during contraction. For example, during 30-second "all-out" sprint interval training (SIT), the pedalling resistance experienced by the individual is typically ~7.5% of body mass (Burgomaster et al., 2008), whereas the average 1-RM leg press can equate to ~170% of body mass (Baar, 2009; Ruiz et al., 2008). Thus, despite the apparently similar work-to-rest ratio between SIT and heavy RE, the mechanical load imparted to the active muscle is vastly different, and appears to be a key mediator of the subsequent adaptations induced in skeletal muscle following repeated training.

2.8 Molecular basis for the concurrent interference effect

Although the molecular signalling mechanisms regulating the specificity of training adaptation are incompletely understood, there appear to be multiple signalling responses induced by endurance exercise capable of inhibiting MPS and stimulating MPB (Figure 2.8). Given muscle fibre hypertrophy requires a positive net balance of MPS above MPB (Atherton & Smith, 2012), the repeated antagonism of these responses by endurance exercise might contribute to limiting fibre hypertrophy following concurrent training (Baar, 2006; Hawley, 2009; Nader, 2006).

Perhaps the most well-characterised such mechanism involves the purported antagonism between the AMPK and mTORC1 signalling cascades (Inoki et al., 2012; Kimball, 2006; Mounier et al., 2011), considered to be predominantly involved in endurance and RT adaptation (Baar, 2006; Coffey & Hawley, 2007), respectively (see section 2.5.2.4). Multiple lines of evidence suggest AMPK activation exerts an inhibitory effect on mTORC1 and its downstream signalling targets, thereby negatively regulating MPS and hypertrophy (Atherton et al., 2005; Bolster et al., 2002; Gwinn et al., 2008; Inoki et al., 2003a; Inoki et al., 2002; Inoki et al., 2003b) (further discussed in

section 2.5.3.4). Accumulating *in vitro* evidence also suggests, in addition to suppressing protein synthesis, AMPK activation stimulates protein degradation via both the ubiquitin-proteasome and autophagy-lysosomal systems (Sanchez et al., 2012a; Sanchez et al., 2012b) (further discussed in sections 2.6.1 and 2.6.2). AMPK activation promotes Fox-O-dependent transcription of Atrogin-1 and MuRF-1 (Nakashima & Yakabe, 2007; Sanchez et al., 2012b; Tong et al., 2009) and disrupts the inhibitory effect of mTORC1 on ULK1 while increasing ULK1 activity, leading to autophagy induction (Jung et al., 2009; Sanchez et al., 2012b). Therefore, the activation of AMPK by endurance exercise potentially mediates interference to skeletal muscle anabolism via down-regulating MPS and concomitantly up-regulating MPB (Coffey & Hawley, 2007).

Protein synthesis is also regulated in the elongation phase of protein translation, which is mediated by elongation factors and is the most energy-consuming stage of protein synthesis (Weigl, 2012). The eukaryotic elongation factor 2 (eEF2) is a critical component of the translational machinery involved in translocation of the ribosome along the mRNA (Kapp & Lorsch, 2004). The eEF2 is phosphorylated (i.e., inactivated) by eEF2 kinase (eEF2K) (Browne & Proud, 2002), which is activated by signalling pathways responsive to increased energy demand or reduced energy supply, such as the AMPK and CaMK pathways, both of which are activated following endurance exercise (Rose et al., 2007; Rose et al., 2006). Conversely, signalling related to RE (i.e., mTORC1 and p70S6K activation) inhibits eEF2K activity *in vitro*, thus releasing its inhibition of eEF2 and increasing translation and protein synthesis rates (Browne et al., 2004; Browne & Proud, 2004; Wang et al., 2001). The activation of eEF2K by endurance exercise is therefore a candidate inhibitor of MPS and, potentially, muscle fibre hypertrophy during concurrent training.

Another upstream inhibitor of mTORC1 and protein synthesis is REDD1 (regulated in DNA damage and development 1) (Kimball et al., 2008; Sofer et al., 2005). REDD1 is activated by a number of metabolic stressors including ATP depletion (Sofer et al., 2005) and hypoxia (Brugarolas et al., 2004; DeYoung et al., 2008; Favier et al., 2010), and is induced by endurance exercise in rat skeletal muscle (Murakami et al., 2011). When activated, REDD1 inhibits mTORC1 indirectly by releasing the inhibition of TSC2 caused by 14-3-3 protein binding (DeYoung et al., 2008; Favier et al., 2010). Overexpression of REDD1 in rodent skeletal muscle has been shown to cause a 10% reduction in muscle fibre size in rodent skeletal muscle (Favier et al.,

2010), and REDD1 expression is associated with muscle atrophy in diabetic mice (Hulmi et al., 2012). The expression of REDD1 mRNA is reduced 3 h following low-intensity RE and blood flow restriction, concomitant with increased mTORC1 mRNA expression (Drummond et al., 2008b). Thus, activation of REDD1 by endurance exercise may be an additional mechanism responsible for inhibiting anabolic responses induced by RE, and subsequently hypertrophy during concurrent training.

The sirtuin (SIRT) deacetylase family of proteins are sensitive to metabolic perturbations including increased NAD^+ and lactate concentrations, and are activated by endurance exercise in skeletal muscle (Philp et al., 2011a). Of the SIRT family expressed in skeletal muscle, SIRT1 has been implicated as a potential regulator of mitochondrial biogenesis, in part because it can regulate the activity of AMPK and PGC-1α (Philp & Schenk, 2013). Of potential relevance to concurrent training, SIRT1 negatively regulates mTORC1 *in vitro* (Ghosh et al., 2010). Activated SIRT1 interacts with and subsequently activates TSC2, thereby down-regulating mTORC1 activity (Ghosh et al., 2010). Increased SIRT1 activity induced by endurance exercise is therefore another potential mechanism by which the mTORC1 pathway and protein synthesis might be suppressed following concurrent exercise.

Collectively, there appears to be multiple signalling responses induced by endurance exercise with the capacity to inhibit components of the translation initiation or elongation machinery, and subsequently rates of protein synthesis. It is important to consider, however, that many of these putative interference mechanisms have been described in cell culture or animal models, and often during non-physiological conditions, which may have limited relevance to human skeletal muscle during exercise (Hamilton & Philp, 2013). Many of these mechanisms are poorly characterised in skeletal muscle, let alone in response to exercise, and further work is required to confirm their relevance to training adaptations in human skeletal muscle. Indeed, few studies have investigated whether these mechanisms appear to operate in humans following concurrent exercise, and therefore potentially contribute to compromised fibre hypertrophy following concurrent training.

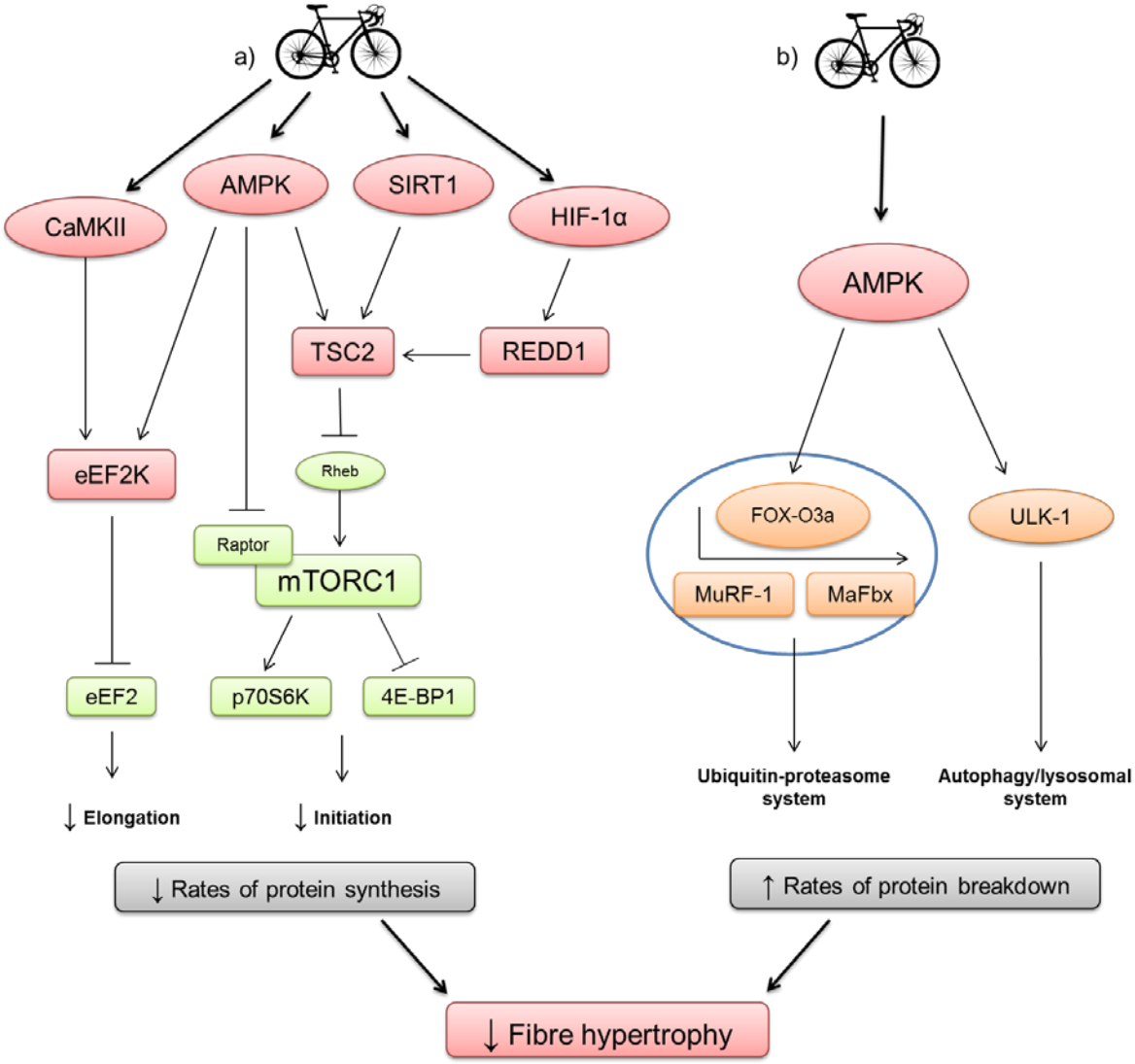

Figure 2.8 Putative molecular mechanisms by which endurance exercise potentially a) inhibits signalling regulating protein synthesis and b) up-regulates pathways mediating protein breakdown, subsequently limiting muscle fibre hypertrophy following concurrent training (Fyfe et al., 2014). CaMKII, calcium/calmodulin-dependent kinase II; AMPK, adenosine monophosphate-activated protein kinase; SIRT1, sirtuin deacetylase 1; HIF-1α, hypoxia-inducible factor-α1; eEF2K, eukaryotic elongation factor 2 kinase; TSC2, tuberous sclerosis complex 2; REDD1, regulated in DNA development and damage 1; eEF2, eukaryotic elongation factor 2; Rheb, Ras homologue enriched in brain; mTORC1, mechanistic target of rapamycin complex 1; p70S6K1, 70 kDa ribosomal s6 protein subunit kinase 1; 4E-BP1, eI4FE binding protein 1; Fox-O3a, forkhead-box O3a; MuRF-1, muscle ring-finger 1; MaFbx, muscle-atrophy f-box (atrogin 1); ULK-1, Unc-51-like kinase 1; ↑, increased/greater; ↓, decreased/less.

2.8.1 Evidence for molecular interference in human skeletal muscle

2.8.1.1 Molecular interference following a single bout of concurrent exercise

A number of human studies have examined molecular responses in skeletal muscle following single bouts of concurrent exercise (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015; Wang et al., 2011) (see Table 2.1 for summary). Most studies have examined skeletal muscle molecular responses in the early post-exercise recovery period (i.e., 15 min to 4 h), and investigated whether these responses are altered with concurrent compared with single-mode exercise (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Lundberg et al., 2012; Pugh et al., 2015; Wang et al., 2011). This approach has provided insight into the existence of 'molecular interference' in humans, and has been used to extrapolate the potential impact of the particular concurrent exercise protocol on chronic training adaptation. Despite numerous efforts to detect molecular interference with concurrent exercise, current evidence is equivocal with regards to the existence of this phenomenon in humans. For example, while some studies have concluded that concurrent training promotes interference of anabolic post-exercise molecular responses in skeletal muscle (Coffey et al., 2009a; Coffey et al., 2009b), others have found that neither protein synthesis rates (Carrithers et al., 2007; Donges et al., 2012) nor mTORC1 signalling (Apro et al., 2015; Apro et al., 2013; Pugh et al., 2015) are different compared with RE alone. There is also evidence concurrent exercise 'potentiates' early adaptive responses to exercise compared to single-mode exercise (Lundberg et al., 2012; Wang et al., 2011). For example, the addition of RE immediately following endurance exercise reportedly augments signalling related to mitochondrial biogenesis (Wang et al., 2011), while performing RE six-hours after endurance exercise enhances mTOR and p70S6K1 phosphorylation (Lundberg et al., 2012) compared with RE alone. Current data therefore provide limited evidence for molecular interference in human skeletal muscle following a single bout of concurrent exercise, and little mechanistic insight into the concurrent training effect. However, as discussed subsequently (see section 2.8.2), the limitations of existing studies must be considered when interpreting evidence with regards to the molecular interference phenomenon in humans. Additionally, given the multitude of variables associated with

concurrent training (e.g., resistance/endurance training modality, volume and intensity of exercise, length of between-mode recovery, nutritional and training status of participants), it is difficult to generalise the findings of existing studies outside of the particular design employed. Further work is therefore needed to examine the role of these additional variables in the concurrent interference effect, while addressing the limitations of current evidence.

Table 2.1 Summary of current evidence regarding molecular interference following a single bout of concurrent resistance and endurance exercise in humans.

Study	Sample size	Participant training status	Study design	Exercise protocols			Results			Conclusions
				Endurance exercise	Resistance exercise	Recovery between modes	Protein phosphorylation	Gene expression	Protein synthesis	
Carrithers et al. (2007)	12 (6 M, 6 F)	Undertaking 1-2 bouts of AE and RE x/wk	Unilateral cross-over. All participants performed unilateral AE followed by bilateral RE	90 min unilateral cycling at 60% W_{peak}	Unilateral leg press and leg extension (3 x 10 repetitions at 80% 1-RM + one set to failure) performed on both legs	30 min	N/A	N/A	Myofibrillar FSR not different between the AE+RE and the RE legs	CE does not suppress post-RE myofibrillar protein synthesis rates independent of muscle glycogen levels
Coffey et al. (2009b)	8 M	Undertaking regular concurrent training (>1 y)	Randomised cross-over. All participants performed AE and RE in alternate orders	30 min continuous cycling at 70% $\dot{V}O_{2peak}$	8 x 5 leg extension repetitions at 80% 1-RM	15 min	↑ p-Akt when RE followed AE. No significant order effect was noted for p-TSC-2/mTOR/p70S6K1	↑ MuRF-1 mRNA when AE followed RE. Reverse order resulted in ↓ IGF-1 mRNA expression	N/A	CE does not promote optimal early signalling responses associated with each mode of exercise
Coffey et al. (2009a)	6 M	Undertaking regular concurrent training (>3 x/wk)	Randomised cross-over. All participants performed both AE and RE in alternate orders	10 x 6-second maximal cycling ergometer sprints against 0.75 Nm $torque^{-1} \cdot kg^{-1}$ (49 sec passive rest between efforts)	8 x 5 leg extension repetitions at 80% 1-RM	15 min	Initial RE ↑ p-p70S6K1 and p-rps6 but this ↓ when RE followed repeated sprints.↑ p-Akt with when RE followed AE. Changes in p-TSC2, p-mTOR and p-AMPK modest and independent of order	↑ MuRF-1 mRNA when sprints followed RE. ↓ IGF-1 mRNA from rest independent of order	N/A	Repeated sprints diminished the anabolic response to RE by attenuating anabolic and enhancing catabolic responses in early recovery

Study	Sample size	Participant training status	Study design	Exercise protocols			Results			Conclusions
				Endurance exercise	Resistance exercise	Recovery between modes	Protein phosphorylation	Gene expression	Protein synthesis	
Wang et al. (2011)	10 (7 M, 3 F)	Not undertaking any programmed exercise for >6 months	Randomised cross-over. All participants performed both AE alone (trial 1) and AE followed by RE (trial 2)	60 min continuous cycling at 65% $\dot{V}O_{2max}$ (3 min rest allowed after 30 min)	Six sets of leg press at 70, 75, 80, 80, 75, 70% 1-RM Participants encouraged to complete maximal repetitions as possible up to 15	15 min	↑ p-mTOR and p70S6K1, ↓ p-eEF2 after AE+RE compared to AE. Similar ↑ in p-Akt, and AMPK in AE+RE and AE. No p-CaMKII after each protocol. Heterogenous p-p38 MAPK response	↑PGC-1α and PDK-4 mRNA with AE+RE compared to AE alone	N/A	Addition of RE after an AE bout ↑ mitochondrial biogenesis and substrate metabolism signalling and may be a novel method for enhancing aerobic capacity
Lundberg et al. (2012)	9 M	Undertaking AE 2-3 x/wk and/or RE 1-2 x/wk for >1 y	Unilateral cross-over. All participants performed unilateral AE followed by bilateral RE 6 hours post-AE	40 min continuous unilateral cycling at 70% of W_{max}. Workload then increased by ~20 W for cycling to exhaustion	Unilateral leg press and leg extension (2 x 7 repetitions of each exercise, 90 sec rest between sets) performed on both legs	6 h	↑ p-mTOR, p-p70S6K1, with AE+RE compared to RE. p-rps6 and p-eEF2 unchanged over time for both legs, trend for ↑ p-rps6 with AE+RE	↑ PGC-1α, VEGF, Atrogin-1 mRNA with AE+RE compared to RE at PRE and 15 min post. ↓ Myostatin levels in AE+RE compared to RE at PRE and 15 min post. MuRF-1 mRNA similar across legs	N/A	Completing RE 6 h after AE did not compromise mTOR-related signalling after leg press and leg extension exercise

Study	Sample size	Participant training status	Study design	Exercise protocols			Results			Conclusions
				Endurance exercise	Resistance exercise	Recovery between modes	Protein phosphorylation	Gene expression	Protein synthesis	
Donges et al. (2012)	8 M	Sedentary middle-aged men (no regular activity involving >30 min/wk for 1 y prior to study)	Repeated measures. All participants completed 3 trials in a randomised order: 1) RE only, 2) AE only and 3) RE+AE combined (50% volume of each isolated session)	40 min continuous cycling at 55% of W_{peak}	8 x 8 leg extension at 70% 1-RM	None	↑ p-Akt at 1 h post in CE compared to RE and AE. No change in p-mTOR, p-p70S6K1, p-AMP, p-MAPK and p-4E-BP1 at any time point. ↑ p-AS160 at 1 and 4 h post for RE. ↑ p-rps6 at 1 h in RE compared to CE and AE	MyoG and MyoD expression ↑ 4 h post RE. MyoG ↑ greater than AE and CE at 1 h post and greater than AE at 4 h post. ↑ MyoD mRNA greater than CE at 1 h post, and AE at 4 h post. No change in myostatin mRNA	Myofibrillar FSR during 4 hr recovery ↑ 1.8 and 2.2-fold for RE and CE trials, respectively. ↑ Myofibrillar FSR for CE/RE both significantly greater than AE which remained unchanged.	CE is as effective as either isolated mode in ↑ myofibrillar and mitochondrial FSR in sedentary middle-aged men despite 50% less training volume of each modality
Apro et al. (2013)	10 M	Undertaking AE 1-2 x/week and RE 2-3 x/wk for >6 months	Randomised cross-over. All participants performed RE followed by AE, or RE alone	30 min cycling at 70% $\dot{V}O_{2max}$.	10 sets of leg press (4 x 8-10 @ 85% 1-RM, 4 x 10-12 @ 75% 1-RM, 2 sets to fatigue at 65% 1-RM)	15 min	↑ p-mTOR and p-p70S6K1 and ↓ p-eEF2 regardless of condition. ↓ p-AMPK and p-ACC regardless of condition	↑ PGC1α mRNA after RE+AE compared to AE	N/A	Endurance exercise performed subsequent to RE does not blunt mTORC1-related signalling
Apro et al.(2015)	8 M	Undertaking AE 1-2 x/week and RE 2-3 x/wk for >6 months	Randomised cross-over. All participants performed AE followed by RE, or RE alone	5 x 4 min cycling at 85% $\dot{V}O_{2max}$.	10 sets of leg press (4 x 8-10 @ 80% 1-RM, 4 x 10-12 @ 70% 1-RM, 2 sets to fatigue at 60% 1-RM)	None	Prior AE ↑ AMPK activity, with no between-trial differences in p-mTOR, p-Akt, p-4E-BP1, p-eEF2, p-p70S6K1 or p70S6K activity after RE with or without prior AE	↑ MuRF-1 and Atrogin-1 mRNA post-exercise for AE+RE vs. RE	No between-trial differences in mixed muscle FSR	Prior high-intensity AE ↑ AMPK but does not interference with mTOR signalling, p70S6K1 activity or mixed muscle FSR after subsequent RE.

Study	**Sample size**	**Participant training status**	**Study design**	**Exercise protocols**			**Results**			**Conclusions**
				Endurance exercise	Resistance exercise	Recovery between modes	Protein phosphorylation	Gene expression	Protein synthesis	
Pugh et al. (2015)	10 M	No structured training for 12 months prior to study	Randomised cross-over. All participants performed RE alone or RE followed by AE	10 x (1 min at 90% HR_{max}, 1 min at 50 W)	4 x 8 leg extension repetitions at 70% 1-RM	None	↑ p-mTOR for RE+AE vs. RE, with no change in p-p70S6K1, rps6, eEF2 or 4E-BP1	↑MuRF-1 and Atrogin-1 and ↓ MyoD and MyoG mRNA for RE+AE vs. RE. ↑ PGC-1α total and splice variant mRNA (ex-1a and ex-1a) after RE+AE vs. RE	N/A	Performing high-intensity AE after RE ↑ p-mTOR compared with RE, but ↑ markers of protein degradation.
Fernandez-Gonzalo et al. (2013)	10 M	Engaged in recreational skiing and team sports 3-5 h/wk. No structured RE training in the past year	Unilateral cross-over. All participants performed unilateral AE followed by bilateral RE 6 hours post-AE, before and after 5 wk training	40 min continuous unilateral cycling at 70% of W_{max}. Workload then increased by ~20 W for cycling to exhaustion	Unilateral leg press and leg extension (2 x 7 repetitions of each exercise, 90 sec rest between sets) performed on both legs	6 h	↑ p-p70S6K1 for AE+RE in untrained, but not trained. No between-leg or pre-post training differences in p-mTOR, p-rps6, p-eEF2	↑ MuRF-1mRNA for AE+RE in untrained, but not trained. ↑ Atrogin-1mRNA for AE+RE in untrained and ↓ Atrogin-1mRNA for AE+RE in trained	N/A	CE ↑ p-p70S6K1 and MuRF-1/Atrogin-1 mRNA vs. RE in untrained, but not trained states.

AE, aerobic exercise; RE, resistance exercise; CE, concurrent exercise; 1-RM, one-repetition maximum; FSR, fractional synthesis rate; ↑, increased/greater; ↓, decreased/less; p, phosphorylation/phosphorylated; W, watts; V̇O2peak, peak oxygen consumption; V̇O2max, maximal oxygen consumption Wpeak/Wmax, peak power output; N/A, data not available; TSC2, tuberous sclerosis complex 2; mTORC1, mechanistic target of rapamycin complex 1; p70S6K1, 70 kDa ribosomal s6 protein subunit kinase 1; MuRF-1, muscle ring-finger 1; mRNA, messenger RNA; Nm, newton-metres; IGF-1, insulin-like growth factor 1; rps6, ribosomal protein s6; VEGF, vascular endothelial growth factor ; AMPK, adenosine monophosphate-activated protein kinase; eEF2, eukaryotic elongation factor 2; CaMKII, calcium/calmodulin-dependent kinase II; MAPK, mitogen-activated protein kinase; AS160, Akt-substrate of 160 kDa; MyoG, myogenin; MyoD, myogenic differentiation 1; ACC, acetyl-CoA carboxylase; PRE, pre-RE; PGC1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PDK, pyruvate dehydrogenase kinase 4.

2.8.1.2 Molecular interference following short-term concurrent training

While limited data exists on molecular responses to single bouts of concurrent exercise, even less information exists regarding the effects of concurrent training on molecular responses to exercise and/or adaptations in skeletal muscle following a period of training (de Souza et al., 2013; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2013). Using a unilateral training model, Lundberg et al. (Lundberg et al., 2013) examined the effect of five weeks of concurrent training vs. RT alone on muscle fibre cross-sectional area (CSA), isokinetic/isometric strength and basal expression of selected regulatory genes (i.e., myostatin, MuRF-1, MaFbx, PGC-1α and VEGF [vascular endothelial growth factor]). Greater quadriceps femoris hypertrophy was observed when RE was preceded by aerobic exercise, compared to RE alone, although no between-limb difference in isometric strength were noted (Lundberg et al., 2013). No differences in the basal expression of selected genes were observed after training. In a subsequent study (Fernandez-Gonzalo et al., 2013), molecular responses in skeletal muscle were examined following concurrent exercise bouts performed before and after the five-week training period of the previous study (Lundberg et al., 2013). Before training, concurrent exercise induced greater post-exercise p70S6K1 phosphorylation and expression of MuRF-1 and Atrogin-1 mRNA compared with RE alone; however, these differences were abolished after training (Fernandez-Gonzalo et al., 2013). Another investigation examined basal phosphorylation and content of selected proteins regulating MPS following eight weeks of concurrent compared with single-mode endurance or RT (de Souza et al., 2013). Despite no between-group differences in measures of muscle strength or quadriceps CSA after training, an increase in basal Akt and AMPK phosphorylation was found for the resistance- and endurance-only groups, respectively, while the concurrent training group showed increased p70S6K1 protein content (de Souza et al., 2013). However, given the exercise protocols employed in these short-term studies were insufficient to cause any interference effect to muscle hypertrophy or strength (de Souza et al., 2013; Lundberg et al., 2013), the interpretation of the observed molecular adaptations in skeletal muscle is difficult. Further work is required to evaluate the effect of long-term concurrent training on skeletal muscle anabolic responses and adaptations to concurrent exercise to provide greater insight into the molecular factors potentially mediating the concurrent training effect.

2.8.2 Limitations of existing molecular concurrent training evidence

There is currently insufficient evidence of molecular interference occurring in human skeletal muscle following a single bout of concurrent exercise to explain the attenuated hypertrophy and strength response following concurrent training. However, it is unclear whether this is a consequence of a lack of the proposed mechanisms operating in human skeletal muscle, and/or the limitations of existing evidence, which are briefly discussed below.

2.8.2.1 Relationship between molecular responses to single bouts of exercise and chronic training adaptations

Most molecular concurrent training studies provide a brief 'snapshot' of the adaptive events occurring in close proximity to single bouts of concurrent exercise (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015; Wang et al., 2011). Many questions remain, however, with regards to the long-term molecular regulation of skeletal muscle adaptations and interference with concurrent training. Firstly, there is limited data indicating a direct coupling between molecular responses to single bouts of exercise and the long-term phenotypic adaptations associated with chronic exercise training (Hawley, 2009; Mitchell et al., 2014). It is therefore unclear whether skeletal muscle molecular responses in the hours following single bouts of concurrent exercise provide a valid indication of the adaptive phenotype, and potential interference, which might be induced if training was repeated long-term. Indeed, the efficacy of the molecular markers commonly used to gauge the anabolic response to exercise and nutritional stimuli has been questioned (Atherton et al., 2010; Phillips et al., 2013), while even rates of MPS following a single bout of RE do not correlate with muscle hypertrophy after 16 weeks of RT (Mitchell et al., 2014). Only four human studies have directly measured protein synthesis rates following single bouts of concurrent exercise (Apro et al., 2015; Camera et al., 2015; Carrithers et al., 2007; Donges et al., 2012), while others have instead utilised proxy markers of MPS and/or MPB (Apro et al., 2013; Coffey et al., 2009a; Coffey et al., 2009b; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Pugh et al., 2015; Wang et al., 2011). Importantly however, a direct coupling between mTORC1 signalling and protein synthesis rates does not always exist in humans

(Atherton et al., 2010), and rates of protein synthesis can be saturated at approximately 30% of the maximal phosphorylation of p70S6K1 in rodents (Crozier et al., 2005). Given these apparent discordances, any minor interference to anabolic signalling responses following single bouts of concurrent exercise may not reflect any potential interference to protein synthesis, and subsequently chronic muscle hypertrophy. Studies extrapolating the anabolic response and potential interference effect from early signalling responses alone must therefore be interpreted with caution. Nevertheless, p70S6K1 phosphorylation following a single bout of RE correlates well (r = 0.82 to 0.99) with chronic hypertrophy in both rodents (Baar & Esser, 1999) and humans (Mayhew et al., 2011; Terzis et al., 2008), supporting this as a proxy marker for chronic hypertrophy and potentially interference with concurrent training. Regardless of the methods used to gauge the post-exercise anabolic response, most concurrent exercise studies have been characterised by limited post-exercise time courses. For example, most existing studies have examined early molecular responses up to 6 h post-exercise, whereas mTORC1 signalling can be sustained for up to 24 h post-exercise (Deldicque et al., 2008; Drummond et al., 2011). These studies may therefore have overlooked any potential effects of concurrent exercise on mTORC1 signalling occurring later than 6 h post-exercise. Further work employing extended post-exercise time-courses is required to determine whether mTORC1 signalling is altered by concurrent exercise during the later recovery period.

2.8.2.2 Effect of training status on early molecular responses to exercise

In addition to the exercise modality, the adaptive state of skeletal muscle appears to impact upon molecular responses to single bouts of exercise (Benziane et al., 2008; Coffey et al., 2006a; Coffey et al., 2006b; McConell et al., 2005; Yu et al., 2003). Both short periods of exercise training (Benziane et al., 2008; McConell et al., 2005; Wilkinson et al., 2008), and years of training in a single exercise modality (Coffey et al., 2006a; Coffey et al., 2006b; Yu et al., 2003), modulate early molecular responses to single bouts of exercise. Merely ten days of endurance training can attenuate AMPK responses to prolonged and sub-maximal intermittent endurance exercise completed at the same absolute pre-training workload (Benziane et al., 2008; McConell et al., 2005). Moreover, while there are little divergences in early mTORC1 signalling responses to single bouts of resistance and endurance exercise in relatively untrained subjects

(Camera et al., 2010; Vissing et al., 2011; Wilkinson et al., 2008), the mTORC1 pathway can be preferentially induced by resistance, but not endurance exercise, in training-accustomed individuals (Vissing et al., 2011). Additionally, while RE performed in the untrained state elicits comparable increases in rates of both myofibrillar and mitochondrial protein synthesis, these responses become more refined following training, whereby only myofibrillar protein synthesis rates are increased in response to RE (Wilkinson et al., 2008). Data from highly strength- or endurance-trained athletes (Coffey et al., 2006a; Coffey et al., 2006b) also supports the notion the skeletal muscle phenotype, rather than the mode of exercise *per se*, can influence the molecular responses to divergent exercise modes. For example, highly-trained endurance cyclists lacked the ability to induce AMPK activation in skeletal muscle following continuous sub-maximal exercise in their habitual discipline (i.e., endurance exercise), while AMPK was activated after exercise in their non-habitual discipline (i.e., RE) (Coffey et al., 2006b). Similarly, an identical endurance training bout performed at the same relative workload was sufficient to activate AMPK in highly-trained powerlifters exhibiting extreme resistance-trained phenotypes (Coffey et al., 2006b). These observations suggest the novelty of the exercise stimulus and associated stressors, not merely the exercise stimulus *per se*, influences early molecular responses to exercise. It therefore appears likely long-term concurrent training would modulate early post-exercise exercise responses (and potentially any interference effect) in skeletal muscle over time, whereby the early molecular profile to unaccustomed exercise bouts may represent a generalised, unrefined adaptive response (Mahoney & Tarnopolsky, 2005; Wilkinson et al., 2008). In this case, evidence regarding molecular responses to single bouts of concurrent exercise in relatively untrained subjects should be interpreted with caution. While most molecular concurrent training studies have utilised participants who were recreationally undertaking both resistance and endurance training (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Lundberg et al., 2012), some have used sedentary participants (Donges et al., 2012; Pugh et al., 2015; Wang et al., 2011) or participants not accustomed to both exercise modalities (Lundberg et al., 2012). Observations that early concurrent exercise bouts promote cumulative effects on protein synthesis and/or mitochondrial biogenesis signalling (Donges et al., 2012; Lundberg et al., 2012; Wang et al., 2011) may therefore be more reflective of the unfamiliarity to the exercise bout (Atherton & Smith, 2012) rather than suggesting enhanced potential for chronic adaptation. Moreover, often

overlooked is that the original concurrent training study by Hickson (1980) showed no detectable interference effect until the eighth week of training, suggesting any interference effect may not manifest until a certain training status is attained. Taken together, these results suggest participant training status is an independent influence on exercise-induced molecular responses in skeletal muscle, and potentially the interference effect, and must be taken into consideration when interpreting existing concurrent training evidence. Future work examining the existence of molecular interference should employ participants who are accustomed to both exercise modes to account for the potentially confounding effects of training status on early post-exercise molecular responses to exercise (Coffey et al., 2006a; Coffey et al., 2006b). Moreover, this further exemplifies the need for longer-term (>8 weeks) training studies examining the potential modulation of interference following periods of concurrent training.

2.8.2.3 Effect of nutrient availability on early molecular responses to exercise

It has become increasingly clear nutrient availability exerts a profound effect on the adaptive responses to exercise training in human skeletal muscle (Beelen et al., 2010; Hawley et al., 2011). For example, the availability of muscle glycogen has been reported to modulate early molecular responses to both endurance and RE in a divergent manner (Cochran et al., 2010; Creer et al., 2005; Yeo et al., 2010). Low carbohydrate availability in close proximity to endurance exercise appears to augment early signalling responses governing skeletal muscle mitochondrial biogenesis and metabolic adaptation (Cochran et al., 2010; Psilander et al., 2012; Yeo et al., 2010), while commencing RE with low muscle glycogen may compromise post-exercise Akt signalling (Creer et al., 2005). Any potential negative effect of low muscle glycogen on anabolic responses in skeletal muscle was, however, recently questioned by a study showing no effect of muscle glycogen depletion on anabolic responses to RE (Camera et al., 2012). Low muscle glycogen is associated with fatigue development (Hulston et al., 2010; Ortenblad et al., 2013) and increased AMPK activity (Derave et al., 2000), which might inhibit anabolic responses induced by RE (Creer et al., 2005; Thomson et al., 2008). As aforementioned (see section 2.5.2.3), amino acids can independently stimulate mTORC1 activation and subsequently increase protein synthesis rates (Blomstrand et al., 2006; Deldicque et al., 2005; Rennie et al., 2006), while branched-chain amino acid provision reduces post-RE increases in Atrogin-1 mRNA and MuRF-1 protein

(Borgenvik et al., 2012). Nutrient availability is therefore a potent modulator of molecular responses to exercise and skeletal muscle adaptations following chronic exercise training (Beelen et al., 2010; Hawley et al., 2011) and must be considered when interpreting the concurrent training literature.

Most existing molecular concurrent training studies have employed designs whereby participants performed exercise in the fasted state (Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Wang et al., 2011), or were not provided with nutrients upon cessation of exercise (Lundberg et al., 2012), presumably to control for the independent effects of nutrient availability on molecular responses within skeletal muscle (Hawley et al., 2011). Performing exercise in the fasted state undoubtedly presents a heightened metabolic challenge within the muscle milieu, presumably increasing energy-sensing kinase activity (e.g., AMPK and eEF2k) with the capacity to suppress protein synthesis (Atherton et al., 2005; Thomson et al., 2008), and promote autophagy (Jamart et al., 2013). The ability of amino acid ingestion to independently stimulate activation of anabolic signalling responses (Blomstrand et al., 2006; Deldicque et al., 2005; Rennie et al., 2006) suggests adequate nutrient availability may be essential for attenuating any potential negative impact of endurance exercise and the associated molecular responses on protein synthesis (Coffey et al., 2011). It is well established that ingestion of sufficient protein in the early recovery period following RE is required to maximise MPS and subsequently muscle hypertrophy (Areta et al., 2013; Hawley et al., 2011). Recent evidence also suggests protein ingestion following concurrent exercise is sufficient to maximise rates of MPS compared with placebo (Camera et al., 2015). Further, as muscle hypertrophy is an energetically-demanding process, a positive energy balance may also be required to support increases in muscle mass (Lambert et al., 2004). Although empirical evidence for this premise is lacking, endurance exercise nevertheless likely disrupts this balance via continual substrate depletion and/or amino acid oxidation (Blomstrand & Saltin, 1999). The potentially confounding effects of altering nutrient availability on molecular responses to exercise should therefore also be considered when interpreting the concurrent training literature. Additionally, further work is required to fully elucidate the importance of nutrient availability on modulating interference during concurrent training (Perez-Schindler et al., 2015).

2.9 The role of concurrent training variables in the interference effect

Given the multitude of potential training variables that may be manipulated in a concurrent training regime, the roles of many of these variables in the interference effect remain incompletely resolved. Specific concurrent training variables might exacerbate molecular interference, either directly by increasing the activity of proteins acting to inhibit MPS and/or stimulate MPB, or indirectly by compromising the 'quality' of the RE stimulus itself (e.g., via residual fatigue or substrate depletion) (Figure 2.9). Improving knowledge of the contribution of these variables to the interference effect is therefore critical to inform their prescription for maximising the simultaneous development of muscle mass, strength and endurance. Existing evidence has nevertheless begun to shed light on the potential roles of specific training variables in the concurrent interference effect.

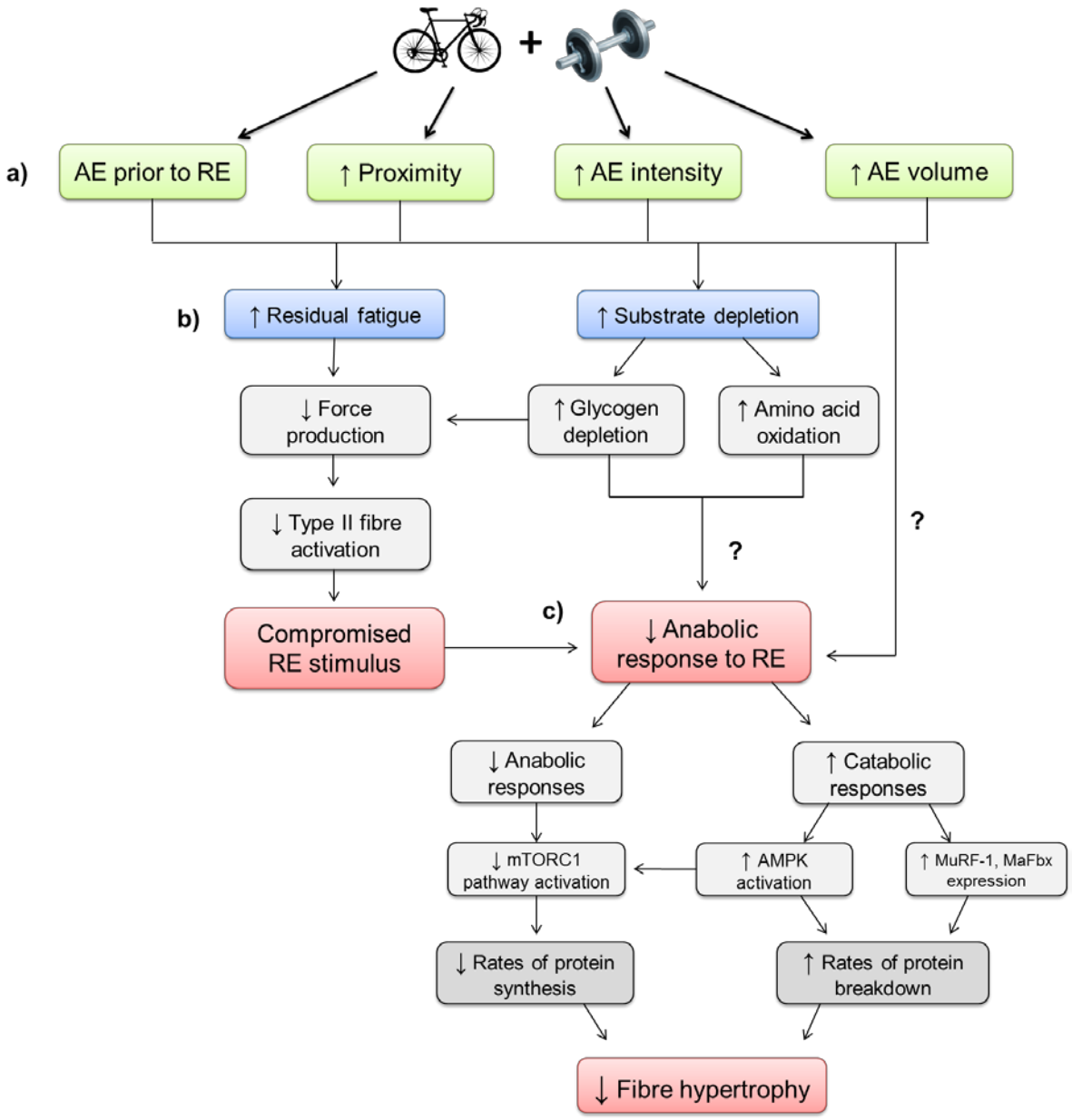

Figure 2.9 Conceptual framework for the potential role of individual concurrent training variables (a) in exacerbating the interference effect, either by compromising the RE stimulus itself via exacerbating residual fatigue and/or substrate depletion (b), or by attenuating the anabolic response to RE (c), subsequently limiting muscle fibre hypertrophy (Fyfe et al., 2014). Proximity, length of recovery allowed between concurrent exercise bouts (i.e, ↑ proximity = ↓ recovery length); AE, aerobic exercise; RE, RE; mTORC1, mechanistic target of rapamycin complex 1; AMPK, adenosine monophosphate-activated protein kinase; eEF2K, eukaryotic elongation factor 2 kinase; MuRF-1, muscle ring-finger 1; MaFbx, muscle-atrophy f-box (Atrogin-1); ↑, increased/greater; ↓, decreased/less.

2.9.1 Within-session exercise order

A common and time-efficient concurrent training approach is to perform divergent exercise bouts together within a single exercise session. The order in which these exercise modes are performed may potentially modulate interference. However, minimal work has been done to ascertain whether an order-effect dependent interference effect exists when concurrently training (Cadore et al., 2012a; Cadore et al., 2012b; Cadore et al., 2012c; Chtara et al., 2008; Collins & Snow, 1993; Gravelle & Blessing, 2000). From a first-principles perspective, it would appear that if substrate depletion and/or residual fatigue (Craig et al., 1991; Leveritt et al., 1999) from prior endurance training bouts compromise performance during subsequent RE, then undertaking RE prior to endurance exercise may alleviate these negative residual effects. However, given metabolic signalling responses to endurance exercise that may inhibit protein synthesis (e.g., AMPK activation) are generally relatively transient (<3 h) (Lee-Young et al., 2008; Wojtaszewski et al., 2003) compared with anabolic responses (i.e., mTOR and p70S6K1 phosphorylation) to RE (>24 h) (Deldicque et al., 2008; Drummond et al., 2011), performing RE after endurance exercise may allow these anabolic responses to proceed unimpeded during early recovery. Nevertheless, current performance-based evidence suggests the effect of within-session exercise order on the interference effect may be limited (Chtara et al., 2008; Collins & Snow, 1993; Gravelle & Blessing, 2000), although performing RE prior to endurance exercise appears to augment neuromuscular and cardiorespiratory adaptations in the elderly (Cadore et al., 2012a; Cadore et al., 2012c). In youth soccer players, performing soccer-specific endurance training prior to RT resulted in greater effect sizes for gains in 1-RM half-squat and various isokinetic strength measures compared with the alternate exercise order (Enright et al., 2015). However, the group performing endurance prior to RT also had an increased duration of between-mode recovery (120 min vs. 30-45 min), as well as different patterns of nutrient provision in close proximity to exercise. Whether these factors may have contributed to the divergent between-group adaptations observed was therefore unclear.

Two similar molecular-based studies (Coffey et al., 2009a; Coffey et al., 2009b) addressed the question of concurrent exercise order by examining early post-exercise signalling responses and gene expression in skeletal muscle after consecutive endurance and RE sessions completed in an alternating order (i.e., resistance prior to endurance exercise, and vice versa). The initial study (Coffey et al., 2009b) employed

consecutive leg extension exercise (8 x 5 repetitions at 80% 1-RM) and continuous cycling (30 min at 70% $\dot{V}O_{2peak}$) and noted a greater increase in MuRF-1 mRNA when cycling followed RE, while the reverse order caused increased phosphorylation of Akt and decreased IGF-1 [insulin-like growth factor 1] mRNA expression (Coffey et al., 2009b). No significant order effect was noted for TSC-2/mTOR/p70S6K1 activation and for the modest increases in PGC-1α mRNA expression. Taken together, the results provided little evidence for any order-effect dependent molecular interference, while the authors suggested concurrent exercise did not promote optimal early signalling responses associated with each exercise mode (Coffey et al., 2009b). However, the lack of a single-exercise mode condition within this design (Coffey et al., 2009b) makes it difficult to speculate on the magnitude of any potential interference relative to RE alone. A subsequent study from the same group (Coffey et al., 2009a) incorporated an identical RE session as the first (Coffey et al., 2009b), but instead combined this with a repeated-sprint cycling protocol (10 x 6-s sprints) performed either before or after RE. These workers reported concurrent repeated-sprint and RE promotes interference by attenuating translation initiation signalling (i.e., p70S6K1 and its downstream target, rps6 [ribosomal protein s6]), and increasing the mRNA abundance of mediators of MPB (i.e., MuRF-1). Divergent p70S6K1 phosphorylation responses were noted between exercise modes and with alternate exercise orders. Specifically, initial RE promoted increased p70S6K1 activation, while this response was attenuated when RE was performed after repeated sprints (Coffey et al., 2009a). These authors concluded performing repeated-sprint exercise in close proximity to RE attenuated anabolic signalling and increased catabolic activity, which likely represents interference to pathways governing RT-related adaptation (Coffey et al., 2009a). Consequently, it was recommended both exercise modes be performed with a significant intervening recovery period to minimise any interference effect, and RT precede repeated sprints if performed within the same session.

2.9.2 Between-mode recovery length

Given performing concurrent exercise bouts in close proximity may represent a sub-optimal training scenario (Coffey et al., 2009a; Coffey et al., 2009b), the recovery length allowed between undertaking concurrent exercise sessions is another important practical consideration. Potentially, residual fatigue and/or substrate (i.e., muscle

glycogen) depletion from endurance training bouts may impact negatively upon both force/power production (Craig et al., 1991; Leveritt et al., 1999) and anabolic signalling responses (Creer et al., 2005) to subsequent RE, respectively. For example, following a bout of endurance exercise, force production of the exercised musculature is reduced for at least 6 hours (Bentley et al., 2000; Bentley et al., 1998; Leveritt & Abernethy, 1999; Sporer & Wenger, 2003), returning to baseline by 24 hours post-exercise (Bentley et al., 1998). Compromised force/power production during RE would theoretically limit activation of higher-threshold (i.e., type IIx) motor units and fibres (Henneman, 1957; Sale, 1987) most susceptible to load-induced hypertrophy (Tesch, 1988). Indeed, the phosphorylation of p70S6K1 (Koopman et al., 2006; Tannerstedt et al., 2009) and mTORC1 (Parkington et al., 2003) following RE is more pronounced in type II compared to type I muscle fibres. Residual fatigue from prior endurance exercise also reduces the volume of work performed during subsequent RE (de Souza et al., 2007; Sporer & Wenger, 2003; Tan et al., 2014), presumably limiting the potential for muscle hypertrophy. Finally, the transient activation of various proteins by endurance exercise that inhibit MPS (e.g., AMPK and eEF2k) (Rose et al., 2009a; Thomson et al., 2008) and mediate MPB (e.g., MaFbx and MuRF-1) suggests commencing RE in closer proximity to endurance exercise may further compromise the anabolic response to RE. Allowing adequate recovery between concurrent exercise sessions may therefore attenuate any negative residual effects from endurance exercise on subsequent training bouts, consequently alleviating any interference. This approach also provides opportunity for carbohydrate and/or amino acid ingestion, essential to replenish muscle glycogen stores (Jentjens & Jeukendrup, 2003) and counteract the detrimental impact of endurance exercise and associated molecular responses on protein synthesis (Atherton et al., 2005; Atherton & Rennie, 2006; Thomson et al., 2008), respectively.

The concept of between-mode recovery has been significantly under-researched in the concurrent training literature (Alves et al., 2015; Sale et al., 1990; Wilson et al., 2012). In their meta-analysis of concurrent training literature, Wilson and colleagues (Wilson et al., 2012) noted a trend (non-statistically significant) towards greater hypertrophy gains in concurrent training studies when resistance and endurance training were performed on separate days compared with on the same day. Early work by Sale and colleagues (1990) found 8 weeks of same-day concurrent training resulted in lower 1-RM leg press strength gain, but not muscle fibre hypertrophy (i.e., knee flexor and extensor CSA), compared with alternate-day concurrent training. Another

investigation (Alves et al., 2015) compared the effects of 8 weeks of concurrent training performed either on the same day or alternate days in prepubescent children. Alternate day concurrent training resulted in greater improvements in 1 kg medicine ball throw distance, $\dot{V}O_{2max}$, and CMJ height, whereas same-day training promoted better improvements in 3 kg medicine ball throw and standing long jump distance. Despite these mixed between-group results, it was suggested concurrent training performed on separate days was optimal for enhancing explosive strength development and $\dot{V}O_{2max}$ in prepubescent children (Alves et al., 2015).

Most existing molecular concurrent training studies have employed designs whereby concurrent resistance and endurance training bouts are performed in succession following only a brief recovery period (e.g., 15-30 min) (Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Wang et al., 2011). These studies therefore provide little mechanistic insight into the effect of extended between-mode recoveries on both force/power production during RE and post-exercise anabolic responses in skeletal muscle. Work by Lundberg et al. (2012) examined early molecular responses to RE (2 x 7 bilateral leg press and leg extension repetitions) performed 6 hours after aerobic exercise (40 min of continuous unilateral cycling at 70% W_{max} [peak power output]) compared to that seen after RE alone. These authors noted divergent exercise modes performed after a significant intervening recovery period in the fed state resulted in elevated anabolic signalling (i.e., increased mTORC1 and p70S6K1 phosphorylation) and lowered myostatin gene expression compared to RE alone. The addition of RE also increased early post-exercise PGC-1α mRNA abundance, while prior endurance exercise did not compromise force and power production during RE. It was therefore concluded divergent exercise modes can be successfully performed on the same day without compromising performance or the molecular responses mediating protein synthesis and mitochondrial biogenesis (Lundberg et al., 2012). However, whether shorter recovery lengths would have exacerbated any putative molecular interference is unclear. Further work is therefore required to determine the role of between-mode recovery in concurrent interference, in addition to recovery strategies that may be employed during this period. Such information may help to develop practical training recommendations for the structuring of concurrent resistance and endurance exercise sessions to support maximal simultaneous development of resistance and endurance training adaptation.

2.9.3 Endurance training volume

The possibility exists the total volume of endurance exercise may be a critical factor in mediating interference during concurrent training (Jones et al., 2013; Ronnestad et al., 2012; Wilson et al., 2012). A role for volume-dependent interference is supported by studies reporting no interference with smaller endurance training frequencies (≤2 sessions per week) (Glowacki et al., 2004; Hakkinen et al., 2003; McCarthy et al., 2002), while others have observed attenuated maximal strength with larger endurance training volumes (≥3 sessions per week) (Bell et al., 2000; Hennessy & Watson, 1994; Hickson, 1980; Jones et al., 2013; Kraemer et al., 1995). Greater attenuation of strength and hypertrophy (estimated via limb girth) has been shown to occur with greater frequencies of concurrent endurance exercise (3 days per week for each mode) compared to when endurance exercise was performed once per week (Jones et al., 2013). Nevertheless, it remains to be determined whether the total weekly endurance training volume, or the training frequency *per se*, is the more critical factor mediating concurrent interference. If endurance exercise volume is key, low-volume HIT protocols (Burgomaster et al., 2008; Metcalfe et al., 2012) might confer benefit when incorporated into a concurrent training regimen by limiting any potential volume-dependent interference effect, while also offering similar metabolic and performance benefits to traditional endurance exercise (Burgomaster et al., 2008; Gibala et al., 2012). Further work in this area is required to inform the manipulation of concurrent endurance training volumes and/or intensities in order to minimise their potentially negative impact on RT adaptations.

2.9.4 Endurance training modality

The endurance training modality employed in a concurrent training regime may also modulate interference following long-term concurrent training (Leveritt et al., 1999; Wilson et al., 2012). Interestingly, the majority of concurrent training studies reporting an interference effect have incorporated running, and less often cycling, as the endurance training modality (Leveritt et al., 1999; Wilson et al., 2012). It remains unclear what might account for any mode-specific interference effect, although it has been suggested this may relate to the similarity between cycling and many strength outcome measures (Gergley, 2009; Wilson et al., 2012), and/or the greater eccentric muscle damage induced by running compared to cycling (Wilson et al., 2012). Whether

running exercise has the capacity to induce greater catabolic molecular activity in skeletal muscle and/or exacerbate residual neuromuscular fatigue in contrast to cycling, which may in turn exacerbate interference, is currently unclear. Relatively little is known regarding the impact of running exercise on early post-exercise adaptive responses in skeletal muscle compared to cycling. Indeed, no studies performed to date have examined the molecular responses in skeletal muscle to concurrent exercise incorporating running as the endurance training modality. Given the majority of team-sport athletes (e.g., Australian football, soccer, rugby etc.) employ running as the predominant endurance training modality, and the anecdotal popularity of running in recreational concurrent training regimes, further work is required to examine the potential consequences of the endurance exercise modality on molecular interference and subsequent long-term adaptations to concurrent training.

2.9.5 Endurance training intensity

Another practical consideration is the intensity of endurance training employed in a concurrent training regime. Recently there has been increased interest in the efficacy of HIT compared with MICT for improving cardiometabolic risk factors and indices of aerobic exercise performance, including $\dot{V}O_{2max}$. As $\dot{V}O_{2max}$ is considered a key component of endurance performance (Coyle, 1999) and is a strong predictor of mortality (Blair et al., 1996), interventions promoting greater increases in $\dot{V}O_{2max}$ are therefore of critical importance for optimising positive health and performance outcomes to exercise programs. A number of studies have demonstrated greater improvement in $\dot{V}O_{2max}$ following HIT compared with work-matched MICT (Gormley et al., 2008; Grieco et al., 2013; Helgerud et al., 2007; Tjonna et al., 2008; Wisloff et al., 2007), although this finding is non-universal (Edge et al., 2006; Edge et al., 2005) (Table 2.2).

Table 2.2 Summary of current evidence regarding the efficacy of high-intensity interval training (HIT) compared with traditional moderate-intensity continuous training (MICT) for improving indices of aerobic exercise performance and metabolic health.

Study	Sample size	Participant training status	Intervention duration	Exercise modality	Exercise protocols			Results	Conclusions
					HIT	MICT	Work-matched ?		
Edge et al. (2005)	20 F	Active in various recreational team sports	3 d/wk for 5 wk	Cycling	4-10 * 2 min intervals/1 min passive recovery, 120-140% LT	12-30 min, 85-95% LT	Yes	**Graded exercise test** • $\dot{V}O_{2peak}$: ↑ 12% for HIT and ↑ 10% for MICT • LT: ↑ 8% for HIT and ↑ 10% for MICT **RSA test** • Greater ↑ in total work for HIT (13%) vs. MICT (8.5%) • Less work (ES, 1.25) and power (ES, 1.9) decrement for HIT vs. MICT • ↑ Peak power for HIT (7%) and MICT (4.7%)	• Similar ↑ in $\dot{V}O_{2peak}$ and LT for HIT vs. MICT when work-matched • Larger ↑ in RSA performance for HIT vs. MICT
Edge et al. (2006)	16 F	Active in various recreational team sports	3 d/wk for 5 wk	Cycling	4-10 * 2 min intervals/1 min passive recovery, 120-140% LT	12-30 min, 85-95% LT	Yes	**Graded exercise test** • $\dot{V}O_{2peak}$: ↑ 14% for HIT and ↑ 12.6% for MICT • LT: ↑ 7% for HIT and ↑ 10.8% for MICT • Muscle buffer capacity (βm *in vitro*) ↑ 25% for HIT, no change (2%) for MICT	• Similar ↑ in $\dot{V}O_{2peak}$ and LT for HIT vs. MICT when work-matched • Training intensity may be a critical stimulus for improving muscle buffer capacity

Study	**Sample size**	**Participant training status**	**Intervention duration**	**Exercise modality**	**Exercise protocols**			**Results**	**Conclusions**
					HIT	MICT	Work-matched ?		
Helgerud et al. (2007)	40 M	Moderately-trained	3 d/wk for 8 wk	Running	**15/15** 47 * 15 s at 90-95% HRmax/15 s at 70% HRmax **4 x 4 min** 4 x 4 min at 90-95% HRmax/15 s at 70% HRmax	**LSD** 70% HRmax for 45 min **LT** 85% HRmax for 24.25 min	Yes	• $\dot{V}O_{2max}$ ↑ for 15/15 (5.5%) and 4*4 min (7.2%) groups, but not for LSD and LT • All training groups similarly ↑ running economy (7.5-11.7%) • Velocity at LT ↑ similarly in all groups (average 9.6%) • No change in LT for any training group when expressed as % $\dot{V}O_{2max}$	High-intensity training (90-95% HRmax) improved $\dot{V}O_{2max}$ more than lower-intensity MICT training (70-85% HRmax)
Tjonna et al. (2008)	13 M, 15 F	Metabolic syndrome patients	3 d/wk for 16 wk	Walking/ running	4 x 4 min at 90% HRmax/3 min at 70% HRmax	70% HRmax for 47 min	Yes	• $\dot{V}O_{2peak}$ ↑ more for HIT (35%) compared with MICT (16%) • Similar ↓ in waist circumference for HIT (5 cm) and MICT (6 cm) • ↑ Insulin sensitivity (HOMA index) for HIT (↑15%) vs. MICT (↓ 14.2%) • 46% (HIT) and 37% (MICT) of participants no longer diagnosed with metabolic syndrome after training	HIT more effective than MICT in reversing features of the metabolic syndrome

Study	Sample size	Participant training status	Intervention duration	Exercise modality	Exercise protocols			Results	Conclusions
					HIT	MICT	Work-matched ?		
Wisloff et al. (2007)	20 M, 7 F	Heart failure patients (age: 75.5 ± 11.1 y)	3 d/wk for 12 wk	Walking	4 x 4 min at 90-95% HRmax/3 min at 50-70% HRmax	70-75% HRmax for 47 min	Yes	• $\dot{V}O_{2peak}$ ↑ more for HIT (46%) compared with MICT (14%) • Anaerobic threshold (% $\dot{V}O_{2peak}$) improved more in MICT than HIT • Improved work economy for HIT but not MICT	HIT is more effective than MICT for improving aerobic capacity, left ventricular remodelling, and endothelial function in elderly patients with chronic heart failure
Gormley et al. (2008)	61 M/F	Healthy (age range: 18-44 y)	3-4 d/wk for 6 wk	Cycling	**Near-maximal intensity** 5 x (5 min at 90-100% HRR, 5 min at 50% HRR)	**Moderate intensity** (50% HRR, 45-60 min) **Vigorous intensity** (75% HRR, 40 min)	Yes	$\dot{V}O_{2peak}$ ↑ more for near-maximal intensity (20.6%), compared with vigorous (14.3%) and moderate (10%) intensities	Higher training intensities elicit greater improvements in $\dot{V}O_{2peak}$ than lower training intensities over a 4-6 wk period in healthy, young adults
Grieco et al. (2013)	19 M, 26 F	Healthy (age: 22.2 ± 3.9 y)	3-4 d/wk for 6 wk	Cycling	**Maximal intensity** 5 x (5 min at 90-100% HRR, 5 min at 50% HRR)	**Moderate intensity** (50% HRR, 45-60 min) **Vigorous intensity** (75% HRR, 40 min)	Yes	• $\dot{V}O_{2peak}$ ↑ for vigorous (15.4%) and maximal (14.2%) intensities, but not moderate intensity (5.8%) • No change in markers of insulin sensitivity or insulin resistance after training	• Higher-intensity exercise more effective than volume-matched lower-intensity exercise for increasing $\dot{V}O_{2peak}$ • No relationship between exercise intensity and insulin sensitivity

Study	**Sample size**	**Participant training status**	**Intervention duration**	**Exercise modality**	**Exercise protocols**			**Results**	**Conclusions**
					HIT	MICT	Work-matched ?		
Berger et al. (2006)	11 M, 12 F	Healthy untrained (no regular exercise for 2 y prior to study)	3-4 d/wk for 6 wk	Cycling	**HI** 20 x (1 min at 90% $\dot{V}O_{2peak}$, 1 min passive recovery) **CON** No exercise	**LO** 30 min at 60% $\dot{V}O_{2peak}$	Yes	• Similar ↑ in $\dot{V}O_{2peak}$ for HI (~20%) and LO (~21%) • Similar ↑ in $\dot{V}O_2$ on-kinetics at onset of moderate- and severe-intensity step exercise for HI and LO	Continuous moderate-intensity exercise as effective as high-intensity exercise for ↑ $\dot{V}O_{2peak}$ and $\dot{V}O_2$ on-kinetics are likely to improve exercise tolerance in untrained populations

HIT, high-intensity interval training; MICT, moderate-intensity continuous training; $\dot{V}O_{2peak}$, maximal oxygen consumption; $\dot{V}O_2$, oxygen consumption; RSA, repeated-sprint ability; HI, high-intensity; LO, low-intensity; CON, control; HRR, heart rate reserve; LT, lactate threshold; ES, effect size; LSD, long slow distance training.

A recent meta-analysis on studies comparing HIT with MICT exercise in patients with lifestyle-induced cardiometabolic disease (Weston et al., 2014a) concluded the average training-induced improvement in $\dot{V}O_{2peak}$ values for the included studies were 19.4% and 10.3% for HIT and MICT, respectively. Taken together, these data suggest HIT is a potent endurance training modality for improving $\dot{V}O_{2max}$ across a variety of populations, including both healthy individuals and those presenting with cardiometabolic risk factors.

Perceived enjoyment of exercise has important implications for long-term adherence to exercise programs. The capacity for exercise interventions to promote perceptions of enjoyment should therefore be considered in addition to its efficacy for promoting positive health and performance outcomes. To this end, it was shown that ratings of perceived enjoyment, as measured by the Physical Activity Enjoyment Scale, were higher after HIT running (6 x 3 min at 90% $\dot{V}O_{2max}$ interspersed with 3 min at 50% $\dot{V}O_{2max}$) compared with MICT running (50 min at 70% $\dot{V}O_{2max}$) (Bartlett et al., 2011). This occurred despite ratings of perceived exertion also being higher during and after the high-intensity interval protocol. Given these protocols were matched for duration, distance run, average intensity, oxygen consumption, and energy expenditure, the observed divergences in perceived enjoyment between exercise protocols likely reflects the varied activity profile rather than any differences in exercise duration (Bartlett et al., 2011). Thus, on a work- and duration-matched basis, it appears HIT may be more favourable compared with MICT with regards to perceived exercise enjoyment, which may translate to better long-term exercise adherence.

In addition to performance outcomes, HIT has emerged as a potent stimulus for inducing signalling in skeletal muscle related to mitochondrial biogenesis (e.g., AMPK phosphorylation and PGC-1α gene expression) compared with MICT (Bartlett et al., 2012; Burgomaster et al., 2008; Gibala et al., 2012; Gibala et al., 2009; Little et al., 2011a; Little et al., 2011b; Little et al., 2010; Psilander et al., 2012). Therefore, HIT may represent a time-efficient strategy for promoting mitochondrial biogenesis and associated improvements in oxidative capacity and metabolic health. This high-intensity approach is also favoured in conditioning programs tailored for enhancing anaerobic capacity and/or repeated sprint ability (Edge et al., 2005; Helgerud et al., 2011). Despite the relevance of HIT for health and performance outcomes (Gibala et al., 2012), little is currently known regarding the effects of incorporating HIT in a concurrent training regime on molecular interference and subsequent adaptation to long-term concurrent

training. Studies independently examining the effect of endurance exercise intensity on concurrent interference are indeed scarce (Silva et al., 2012). One study suggested no role for endurance exercise intensity on interference in physically-active females (Silva et al., 2012); however, training volume and frequency were comparably low and may have limited any potential interference effect seen with higher training volumes (Jones et al., 2013; Ronnestad et al., 2012; Wilson et al., 2012). Moreover, the endurance training protocols employed (Silva et al., 2012) were matched for total exercise duration, and not total work, making it difficult to deduce any potential influence of training intensity in mediating any effect on training-induced maximal strength outcomes. Further work is therefore required to delineate the potential roles of endurance training intensity on interference to maximal strength, power and hypertrophy outcomes during concurrent training. Most existing molecular concurrent training research has employed low-to-moderate intensity endurance exercise protocols (e.g., 30-60 min at 65-70% $\dot{V}O_{2max}$, 40-90 min at 55-70% W_{max}) (Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009b; Donges et al., 2012; Lundberg et al., 2012; Wang et al., 2011), while none have directly examined the effect of endurance exercise intensity on early molecular and chronic performance interference. Coffey and colleagues (2009a) observed that repeated maximal 6-s cycling sprints attenuated anabolic post-exercise responses when performed concurrently with RE (Coffey et al., 2009a), and possibly more so than in a previous study employing moderate-intensity continuous cycling (Coffey et al., 2009b). However, little is known regarding the role of more practical HIT models, such as those involving longer work intervals interspersed with periods of active and passive recovery (Bartlett et al., 2011), on chronic training adaptations when performed concurrently with RE. Two studies have recently shed further light on the impact of HIT on early molecular responses to concurrent compared with RE alone (Apro et al., 2015; Pugh et al., 2015). In a comprehensive study (Apro et al., 2015), performing HIT immediately prior to RE increased post-exercise AMPK activity; however, it did not interfere with mTORC1 signalling responses, rates of mixed MPS, or p70S6K1 kinase activity. A separate study also showed performing HIT immediately after RE also does not interfere with mTORC1 signalling responses up to 6 h post-exercise in untrained individuals (Pugh et al., 2015). However, both of these studies (Apro et al., 2015; Pugh et al., 2015) noted concurrent HIT and RE exacerbated the expression of MuRF-1 and Atrogin-1 mRNA, potentially indicating increased rates of protein degradation. Further work is however required to determine the influence of

HIT on molecular responses to concurrent exercise when compared to MICT, and particularly the impact of these protocols on long-term adaptations to maximal strength, power, and body composition, to elucidate the role of endurance training intensity in mediating the interference phenomenon.

From a molecular perspective, higher-intensity endurance exercise (i.e., HIT) may be expected to exacerbate early molecular interference when compared with lower-intensity (i.e., continuous) endurance exercise. For example, selected negative regulators of protein synthesis, such as AMPK and 4E-BP1, are up-regulated by endurance exercise in an intensity-dependent manner (Chen et al., 2000; Rose et al., 2009b; Rose et al., 2006). Moreover, the AMPK-α1 catalytic isoform, which purportedly plays a selective role in mTORC1 inhibition (McGee et al., 2008a; Mounier et al., 2009), may be preferentially activated at higher (Chen et al., 2000), but not lower (Wojtaszewski et al., 2002), endurance exercise intensities. Higher-intensity endurance exercise also appears to inhibit subsequent force production (Bentley et al., 2000; Leveritt & Abernethy, 1999), while lower-intensity continuous exercise may cause less residual fatigue (Leveritt et al., 2000). Finally, higher exercise intensities are associated with increased glycogen depletion occurring predominantly in type II muscle fibres (Gollnick et al., 1974), which may exacerbate residual fatigue (Hulston et al., 2010) and increase inhibitory AMPK activity (Derave et al., 2000). Whether the capacity of higher-intensity exercise to cause greater metabolic perturbation in type II muscle fibres (Gollnick et al., 1974; Thomson et al., 1979) plays any role in potentially blunting anabolic responses within these fibres following subsequent RE remains to be determined.

Notwithstanding the relevance of high-intensity exercise for improving markers of aerobic exercise performance and metabolic health, in addition to its implications for exercise enjoyment and adherence, little attention has been paid to the incorporation of divergent endurance training intensities into concurrent training programs. Only a single study has to date incorporated concurrent training groups performing endurance exercise of different endurance training intensities (Silva et al., 2012). It is therefore unclear whether HIT represents a more favourable exercise strategy compared with MICT, from the perspective of inducing positive adaptations to exercise performance and metabolic health when incorporated into concurrent training programs.

2.10 Conclusion and future directions

While considerable performance-based evidence exists for the concurrent interference effect, limited data is available regarding both the molecular bases and the role of specific training variables in this phenomenon. Findings from existing research are complicated by the multitude of potential concurrent training variables and the numerous independent factors capable of influencing early post-exercise molecular responses in human skeletal muscle. There is substantial difficulty, therefore, in deducing practical training recommendations from existing research for minimising interference during concurrent training. Given the growing evidence base for the potency of HIT for improving markers of metabolic health and performance compared with traditional MICT, endurance training intensity is a particularly important and under-researched training variable in the concurrent training literature. Whether the intensity of endurance training incorporated into a concurrent training regime is an important mediating factor for any chronic interference effect is therefore currently unknown. Despite much recent interest, a molecular basis for the phenomenon of attenuated RT adaptations with concurrent training remains elusive. While considerable advances have been made with regards to our understanding of the molecular factors mediating training adaptation in skeletal muscle, these complex processes are incompletely resolved. The possibility remains that current analytical techniques solely encompassing measures of intramuscular signalling and/or protein synthesis with poor temporal resolution has limited our understanding of the factors mediating exercise-induced adaptation in skeletal muscle. Recent information has emerged regarding the epigenetic regulation of both transcriptional and translational processes (e.g., by miRNAs) likely to provide further insight into exercise-induced training adaptation, and potentially the concurrent interference phenomenon. There is also growing evidence that increases in translational capacity (i.e., ribosome biogenesis) accompany muscle hypertrophy consequent to RT, and markers of ribosome biogenesis are attenuated in situations where muscle mass might be compromised (e.g., in ageing or with chronic inflammation). However, to date the majority of concurrent training literature has focused solely on the hypothesis that attenuated muscle hypertrophy may be mediated via post-exercise attenuation of translation initiation signalling (i.e., mTORC1 signalling) and rates of MPS. The effects of concurrent exercise compared with single-mode RE on both skeletal muscle miRNA expression and ribosome biogenesis therefore

remains unknown. In summary, further work is required to elucidate the roles of various concurrent training variables in the interference effect to inform practical recommendations for minimising interference following long-term concurrent training. There is also a need to investigate novel molecular mechanisms implicated in skeletal muscle adaptation to exercise in the context of concurrent training, particularly in training-accustomed individuals, to provide further mechanistic insight into the interference effect.

Chapter 3. Effect of concurrent exercise incorporating high-intensity interval or work-matched continuous training on post-exercise mTORC1 signalling and microRNA expression in human skeletal muscle

Adapted from: Fyfe, J. J., Bishop, D. J., Zacharewicz, E., Russell, A. P., & Stepto, N. K. (2016). Concurrent exercise incorporating high-intensity interval or continuous training modulates mTORC1 signalling and microRNA expression in human skeletal muscle. *American Journal of Physiology, Regulatory, Integrative and Comparitive Physiology*. Jun 1;310(11):R1297-311.

3.1 Linking statement

As outlined in Chapter 2, evidence gleaned from experiments in rodent skeletal muscle and *in vitro* suggests activation of the AMPK pathway, among others, can inhibit the activity of the mTORC1 pathway. Work conducted in humans has attempted to elucidate whether inhibition of mTORC1 signalling following concurrent exercise might explain attenuated muscle hypertrophy responses with concurrent training. Human studies have, however, failed to observe any interference to mTORC1 signalling or rates of MPS with concurrent training compared with RE performed alone. Despite these observations, as discussed in Chapter 2, the potential role of individual training variables in mediating any interference effect to early molecular responses after concurrent exercise has received little attention in the literature. Given the potency of HIT for improving performance and markers of metabolic health compared with traditional MICT, along with its potential for long-term exercise adherence, endurance training intensity is a particularly important, yet overlooked, practical consideration with concurrent training. Most studies investigated early molecular responses to concurrent training have employed MICT, with only two recent studies (Apro et al., 2015; Pugh et al., 2015) incorporating HIT. However, to fully elucidate the potential role of endurance training intensity in modulating any interference effect following concurrent exercise, direct comparisons between concurrent exercise bouts incorporating divergent endurance exercise intensities are necessary. Moreover, novel potential mediators of exercise-induced adaptations in skeletal muscle, including microRNAs, are of great interest and have not yet been investigated in the context of concurrent exercise. The aim of the following chapter was therefore to investigate the potential role of endurance training intensity in mediating interference to mTORC1 signalling, and the expression of miRNAs in skeletal muscle purported to regulate pathways involved with protein synthesis and/or myogenesis.

3.2 Introduction

Incorporating both resistance (RE) and endurance exercise into a periodised training program is termed concurrent training (Leveritt et al., 1999). Compared with undertaking RE alone, concurrent training attenuates skeletal muscle hypertrophy and maximal strength development in some (Bell et al., 2000; Chtara et al., 2008; Dudley & Djamil, 1985; Hickson, 1980; Kraemer et al., 1995), but not all (Balabinis et al., 2003; Lundberg et al., 2013; McCarthy et al., 2002; Sillanpaa et al., 2009; Silva et al., 2012), studies. Given that skeletal muscle mass plays an important role in overall metabolic health (Wolfe, 2006), and many athletes require elements of strength and muscle hypertrophy, concomitantly with a high aerobic capacity (Helgerud et al., 2011), minimizing interference during concurrent training has implications for optimizing both health and performance outcomes.

The mechanistic target of rapamycin complex 1 (mTORC1) is a key mediator of load-induced increases in muscle protein synthesis (MPS) (Bodine et al., 2001b; Drummond et al., 2009). The activity of mTORC1 is antagonised by activation of the 5' adenosine monophosphate-activated protein kinase (AMPK), which acts to restore perturbations in cellular energy balance by inhibiting anabolic cellular processes, such as protein synthesis, and stimulating catabolism (Kimball, 2006). Given that AMPK, acting primarily through the peroxisome proliferator-activated gamma receptor co-activator 1α (PGC-1α), has been associated with many skeletal muscle adaptations to chronic endurance training (Olesen et al., 2010), the interference effect with concurrent training may be partly explained by AMPK-mediated inhibition of the mTORC1 signalling pathway. However, several human studies have shown that concurrent exercise does not compromise either post-exercise mTORC1 signalling or rates of MPS (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Donges et al., 2012; Pugh et al., 2015), or even potentiates these responses (Lundberg et al., 2012), relative to RE performed alone. Whether these putative interference mechanisms explain the attenuated skeletal muscle adaptations reported following concurrent training in humans (Bell et al., 2000; Kraemer et al., 1995; Wilson et al., 2012) remains unclear.

Additional molecular mechanisms with the potential to regulate skeletal muscle adaptations to exercise have recently emerged, including altered microRNA (miRNA) expression (Zacharewicz et al., 2013). miRNAs are small (~20-30 nucleotides in length), non-coding ribonucleic acid (RNA) species that are highly expressed in skeletal

muscle (Zacharewicz et al., 2013). The primary function of miRNAs is to decrease protein levels either by repressing gene translation or promoting the degradation of target mRNAs, of which the latter accounts for the majority of miRNA activity (Guo et al., 2010). Given their purported role in post-transcriptional regulation, miRNAs have emerged as a potential regulator of exercise-induced adaptations in skeletal muscle (Zacharewicz et al., 2013). The expression of various miRNA species in human skeletal muscle is altered following both single bouts of exercise (Drummond et al., 2008c; Russell et al., 2013; Zacharewicz et al., 2014) and after short-term exercise training (Davidsen et al., 2011; Russell et al., 2013). miRNAs have been implicated in the regulation of the IGF-1/Akt, Fox-O1, and myogenesis pathways, all known to play a role in RE-induced adaptations in skeletal muscle (Hitachi & Tsuchida, 2013), although the direct impact of miRNAs on these pathways in response to RE is yet to be experimentally validated. In addition, whether miRNAs are differentially regulated following concurrent exercise, compared with single-mode RE, is currently unknown.

From a practical standpoint, elucidation of the roles of specific training variables (e.g., training intensity, volume, and modality) is required to inform exercise prescription guidelines for minimizing potential interference during concurrent training (Fyfe et al., 2014). One important practical consideration is the intensity of endurance training employed in a concurrent training program. Recent evidence suggests that high-intensity interval training (HIT) is more effective in improving $\dot{V}O2_{max}$ (Gormley et al., 2008; Grieco et al., 2013; Tjonna et al., 2008; Wisloff et al., 2007) and metabolic health markers (Tjonna et al., 2008; Wisloff et al., 2007), compared with moderate-intensity continuous training (MICT), while also being more enjoyable (Bartlett et al., 2011). However, most studies investigating the early molecular responses to concurrent exercise in humans have employed MICT (e.g., 30-90 min at 65-70% $\dot{V}O_{2max}$ or 55-70% W_{max}) (Carrithers et al., 2007; Coffey et al., 2009b; Donges et al., 2012; Lundberg et al., 2012; Wang et al., 2011). Whether the intensity of endurance exercise, when performed concurrently with RE, is important in mediating any potential interference effect on post-exercise anabolic responses in skeletal muscle is unknown. There are several potential mechanisms by which higher-intensity endurance exercise may exacerbate molecular interference during concurrent training, compared to lower-intensity endurance exercise. For example, endurance exercise activates putative inhibitors of MPS, including AMPK and 4E-BP1 (eIF4E binding protein 1), in an intensity-dependent manner, with higher relative exercise intensities associated with

greater AMPK and 4E-BP1 activation (Rose et al., 2009b). Higher-intensity endurance exercise also induces greater muscle glycogen depletion (Gollnick et al., 1974), which is associated with increased AMPK activity (Derave et al., 2000). Taken together, these factors suggest that performing higher-intensity endurance exercise concurrently with RE may be sub-optimal with regards to promoting anabolic responses in skeletal muscle following concurrent exercise. The effect of concurrent exercise incorporating divergent endurance training intensities on early post-exercise anabolic responses in skeletal muscle has not been directly compared.

The aim of this study was to compare the effects of a single bout of concurrent exercise, incorporating either HIT or work-matched MICT cycling, on mTORC1 signalling and miRNA expression in human skeletal muscle, compared with RE performed alone. Between-trial comparisons were made to determine i) the effects of prior endurance exercise on these responses before commencing subsequent RE, ii) whether prior endurance exercise altered mTORC1 signalling and miRNA expression after subsequent RE, compared with RE performed alone, and iii) if these responses were different when RE was performed after HIT compared with MICT. It was hypothesised that, compared with MICT, HIT would further i) increase glycogen depletion and AMPK activity (indexed by AMPK α-subunit and AMPK substrate acetyl-CoA carboxylase [ACC] phosphorylation) prior to subsequent RE, ii) attenuate mTORC1 signalling after subsequent RE, and iii) alter the post-exercise expression of miRNAs linked to the regulation of skeletal muscle adaptations to RE.

3.3 Methodology

3.3.1 Participants

Eleven males participating in regular physical activity incorporating both resistance and endurance exercise (>30 min, 3-5 times per week) were initially recruited for this investigation; however, three participants withdrew due to circumstances unrelated to the study. Eight participants (mean ± SD: age, 27 ± 4 y; height, 178.3 ± 6.1 cm; body mass, 83.7 ± 13.7 kg; peak oxygen uptake [$\dot{V}O_{2peak}$], 45.7 ± 9 $mL \cdot kg^{-1} \cdot min^{-1}$; unilateral 1RM leg press, 173.6 ± 37.1 kg [left leg], 176.4 ± 33.7 kg [right leg]) therefore completed this study. Power calculations using G*Power 3.1 software

indicated a sample size of 9 participants was necessary to detect a moderate effect (*d* = 0.50) for between-trial differences in protein phosphorylation (Coffey et al., 2009b) whereby $\alpha = 0.05$ and $\beta = 0.2$. After being fully informed of study procedures (Appendix A) and screening for possible exclusion criteria (Appendices C and F), participants provided written informed consent (Appendix D). All procedures were approved by the Victoria University Human Research Ethics Committee.

3.3.2 Experimental design

This study followed a within-subject, repeated-measures design. After familiarisation and preliminary testing, participants completed three experimental trials in a randomised order. Each trial was separated by ~1 week and participants were asked to maintain habitual diet and exercise habits during this period. Experimental trials were: 1) RE performed alone (RE trial), 2) HIT cycling followed by RE (HIT+RE trial) or 3) work-matched MICT cycling followed by RE (MICT+RE trial).

3.3.3 Preliminary testing

Preliminary testing was undertaken at least 1 week prior to the first experimental trial to determine the lactate threshold (LT), $\dot{V}O_{2peak}$ and one-repetition maximum (1RM) strength. Preliminary testing data was used to standardise relative exercise intensities during experimental trials with respect to the LT and 1RM. Endurance exercise intensities were standardised relative to the LT rather than $\dot{V}O_{2peak}$, as physiological responses are more consistent, independent of training status, when exercise is undertaken at an intensity relative to LT, compared to $\dot{V}O_{2peak}$ (Baldwin et al., 2000).

3.3.3.1 Graded exercise test (GXT)

The participants' LT and peak power output (W_{peak}) were determined during a graded exercise test (GXT) to volitional exhaustion on an electromagnetically-braked cycle ergometer (Lode, Groningen, The Netherlands). Prior to the GXT, a venous catheter was inserted into an antecubital forearm vein for subsequent blood sampling. The GXT comprised 4-min work stages interspersed with 30 s of passive recovery. Participants were required to maintain a pedalling cadence of 70 rpm during each work

stage. The initial workload was set at 60, 90 or 120 W (to limit the number of stages to a maximum of 10, as determined during familiarisation), and increased by 30 W for each subsequent stage until volitional exhaustion, defined as an inability to maintain a cadence >60 rpm. Venous blood samples (~1 mL) were obtained at rest, and immediately following completion of each work stage. Whole-blood samples were immediately analysed in duplicate for lactate concentration using an automated analyser (YSI 2300 STAT, Yellow Springs, OH). The lactate threshold was defined as the first workload that elicited a >1 mM increase in venous blood lactate concentration (Coyle et al., 1984) and was calculated using Lactate-E version 2.0 software (Newell et al., 2007). The W_{peak} was determined as previously described (Hawley & Noakes, 1992).

3.3.3.2 Peak oxygen uptake ($\dot{V}O_{2peak}$) test

Immediately following the GXT, a 5-min active recovery was initiated at 20 W, after which participants cycled to volitional exhaustion at a workload corresponding to 105% of the W_{peak} achieved during the GXT. Participants were instructed to accelerate to a cadence of 90-100 rpm upon a 5-s countdown, and the test was terminated when a cadence >60 rpm was no longer possible. Expired gases were sampled every 15 s during this test component using automated gas analysers (Moxus Modular $\dot{V}O_2$ System, AEI Technologies, Pittsburgh, PA). A similar protocol has previously been reported to elicit $\dot{V}O_{2peak}$ values no different to that determined during a ramp incremental test performed 5 min previously (Rossiter et al., 2006). The gas analysers and pneumotach were calibrated prior to each test using known gas concentrations (21.0% O_2 and 0.04% CO_2, 16.0% O_2 and 4.0% CO_2) and a 3-L calibration syringe, respectively. The individual $\dot{V}O_{2peak}$ was defined as the highest two consecutive 15-s values achieved during the test.

3.3.3.3 Maximal strength (1RM) testing

Maximal strength was determined during a series of one-repetition maximum (1RM) unilateral leg press attempts using a plate-loaded 45° incline leg press (Hammer Strength Linear, Schiller Park, IL). After a standardised warm-up (5 and 3 repetitions for each leg at 50 and 70% estimated 1RM, respectively), single repetitions of increasing load were attempted for each leg until the maximal load possible for one repetition was determined. The test commenced with the dominant leg, and a one-

minute recovery was allowed before the other leg performed the equivalent 1RM attempt. Three minutes of recovery was allowed between 1RM attempts for the same leg. Participants completed each repetition with the heel placed at the bottom edge of the foot plate, and with a range of motion of 90° knee flexion/extension.

3.3.4 Exercise and dietary control

For 24 h prior to each experimental trial, participants were asked to refrain from strenuous and/or structured physical activity and were provided with a standardised diet. The daily energy intake (~149 $kJ \cdot kg^{-1}$; ~36 $kcal \cdot kg^{-1}$) comprised ~5.1 $g \cdot kg^{-1}$ carbohydrate, ~1.3 $g \cdot kg^{-1}$ protein, and ~1.2 $g \cdot kg^{-1}$ fat. The percentage contribution of each macronutrient to the daily energy intake was 62% carbohydrate, 12% protein and 26% fat. On the morning of each experimental trial, a standardised breakfast (~13 $kJ \cdot kg^{-1}$; ~3.1 $kcal \cdot kg^{-1}$) providing ~0.7 $g \cdot kg^{-1}$ carbohydrate, ~0.1 $g \cdot kg^{-1}$ protein and < 0.01 $g \cdot kg^{-1}$ fat was ingested 90 min before the initial muscle biopsy. The percentage contribution of each macronutrient to the breakfast energy intake was 85% carbohydrate, 13% protein and 2% fat.

3.3.5 Experimental trial overview

On the morning of an experimental trial (Figure 3.1), participants reported to the laboratory at ~7:00 AM after a 10-12 h fast. A venous catheter was then inserted into an antecubital forearm vein for subsequent blood sampling, and a resting blood sample was obtained. Participants then ingested the standardised breakfast (described previously). For the RE trial, participants then rested quietly in the laboratory for 90 min before a resting biopsy was obtained and the RE protocol commenced (described subsequently). For the HIT+RE and MICT+RE trials, participants performed identical procedures as the RE trial, however either the HIT or work-matched MICT protocol (both described subsequently) was completed 15 min prior to RE. After completion of RE, participants rested quietly in the laboratory for 3 h, and additional vastus lateralis biopsies were obtained 1 h and 3 h after completion of RE.

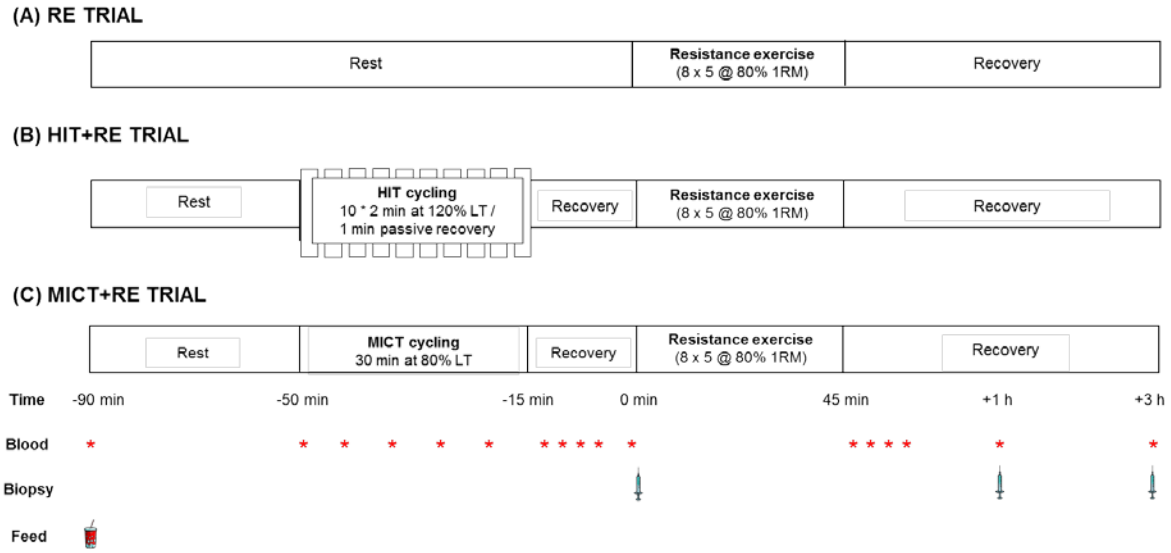

Figure 3.1 Experimental trial timelines for Study 1. For the RE trial (A), participants completed the RE protocol alone. For the HIT+RE (B) and MICT+RE (C) trials, RE was performed after 15-min recovery from either the HIT or work-matched MICT cycling. Muscle biopsies were obtained from the vastus lateralis immediately before RE, either without (PRE) or with (POST) prior endurance exercise, and 1 h and 3 h after completion of RE. HIT, high-intensity interval training; LT, lactate threshold; MICT, moderate-intensity continuous training; 1-RM, one-repetition maximum.

3.3.5.1 Cycling exercise

After a standardised warm-up (5 min at 75 W), participants commenced a bout of either HIT (10 x 2 min at 120 % LT interspersed with 1 min of passive recovery) or work- and duration-matched MICT (30 min at 80% LT) (Edge et al., 2006) (see Figure 1) on an electromagnetically-braked cycle ergometer (Velotron, Racer-Mate, Seattle, WA). Heart rate (Polar FT1, Kempele, Finland) and rating of perceived exertion (RPE) (Borg, 1970) were obtained at regular intervals throughout exercise. Cycling was performed in the seated position and at a fixed cadence of 80 and 100 rpm for MICT and HIT, respectively. Following completion of endurance exercise, participants rested passively for 15 min before undertaking RE.

3.3.5.2 Resistance exercise

After a standardised warm-up (5 and 3 repetitions at 50% and 60% 1RM, respectively) participants completed 8 x 5 unilateral leg press repetitions on each leg at 80% of the 1RM. The biopsied leg first completed each set, and 1-min recovery was

allowed before the opposing leg completed the equivalent set. Three minutes of recovery was allowed between sets for the same leg. Participants completed each repetition with the heel placed at the bottom edge of the foot plate, and with a range of motion of 90° knee flexion/extension. All participants successfully completed the identical resistance exercise protocol for each experimental trial, which was not influenced by prior HIT or MICT.

3.3.5.3 Blood sampling

Venous blood samples (~1 mL) were obtained at rest, immediately pre-cycling and after 10, 16, 22, 28 and 34 min of cycling. Additional blood samples were drawn after 2, 5, 10 and 15 min recovery from cycling, immediately following completion of RE, and after 2, 5, 10, 30, 60, 120 and 180 min of recovery from RE. Blood samples were aliqouted into a microtube (~1 mL) and immediately analysed for glucose and lactate concentrations using an automated analyser (YSI 2300, Yellow Springs, OH).

3.3.5.4 Muscle sampling

Muscle biopsies were obtained from the middle-portion of the *vastus lateralis* using the Bergström technique (Bergstrom, 1962) modified with suction (Evans et al., 1982). After administration of local anaesthesia (1% Xylocaine), three small incisions (~6 mm, ~1 cm apart) were made to the anaesthetised skin in preparation for a series of muscle biopsies. Biopsies were obtained immediately before RE (PRE), and 1 h and 3 h after RE. To minimise the number of biopsy samples taken from participants, the initial resting biopsy obtained from the RE trial was used as a basal reference for changes in signalling responses and gene expression for each trial (Donges et al., 2012). This approach allowed insight into the influence of prior HIT and MICT on responses within skeletal muscle before subsequent RE. All within-trial biopsies were obtained from the same leg, which was alternated for the subsequent trials. Muscle samples were blotted on filter paper to remove excess blood, immediately frozen in liquid nitrogen, and stored at –80°C until subsequent analysis.

3.3.6 Western blotting

Approximately 15 mg of muscle tissue was homogenised in ice-cold lysis buffer (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 20 mM Tris, 1 mM EDTA, 1% Triton X-100, 10% Glycerol, 5 mM Na pyrophosphate, 1 mM NaF, 1µg/mL Leupeptin, 1 mM PMSF, 1µg/mL Aprotinin, 1 mM Na_4VO_3, 1 mM DTT, 1 mM Benzamidine). Samples were rotated end-over-end for 1 h at 4°C, centrifuged at 15,000 *g* for 10 min at 4°C and the supernatant collected. After determination of protein concentration (Bradford assay), the lysate was re-suspended in 2×Laemmli buffer (1.5 M Tris-HCl, 4% SDS, 20% Glycerol, 1.5% Bromophenol blue), and stored at -80°C until subsequent analysis. Depending on the protein target, either 15 or 20 µg of protein was separated by SDS-PAGE using either 6, 8, or 12% acrylamide gels in a 1× running buffer (25 mM Tris, 192 mM Glycine, 0.1% SDS), and then transferred to polyvinylidine fluoride (PVDF) membranes (Bio-Rad laboratories, Hercules, CA) using a wet transfer for 90 min in 1 x transfer buffer (25 mM Tris, 192 mM Glycine, 0.1% SDS, 20% Methanol). After transfer, membranes were blocked for 1 h at room temperature with 7.5% skim milk in 1×TBST (200 mM Tris, 1.5 M NaCl, 0.05% Tween 20), washed with 1×TBST (5×5 min), and incubated with primary antibody solution (5% BSA [Bovine Serum Albumin], 0.05% Na Azide in 1×TBST) overnight at 4°C. Primary antibodies for monoclonal p-mTOR^{Ser2448} (1:1000; #5536), p-p70S6K^{Thr389} (1:1000; #9234), p-4E-BP1$^{Thr37/46}$ (1:1000; #2855), p-AMPK^{Thr172} (1:1000; #2535), p-rps6$^{Ser235/236}$ (1:750; #4856), p-GSK-3α/β$^{Ser21/9}$ (1:1000; #9331), and polyclonal p-eEF2^{Thr56} (1:1000; #2331) and p-ACC^{Ser79} (1:1000, #3661) were from Cell Signalling Technology (Danvers, MA). The following morning, membranes were washed again with 1× TBST and incubated with a secondary antibody (Perkin Elmer, Waltham, MA, #NEF812001EA; 1:5000 or 1:10000 in 5% skim milk and1× TBST) for 1 h at room temperature. After washing again with 1×TBST, proteins were detected with chemiluminescence (Clarity™ Western ECL Substrate, Bio-Rad Laboratories, Hercules, CA) using a VersaDoc™ 4000 MP imaging system (Bio-Rad laboratories, Hercules, CA and quantified via densitometry (Image Lab 5.0, Bio-Rad laboratories, Hercules, CA). All sample timepoints for each participant were run on the same gel and normalised to both an internal pooled sample present on each gel and the total protein content of each lane using a modified Coomassie staining protocol (Welinder & Ekblad, 2011). Briefly, after imaging, membranes were washed in 1×TBST, stained with 0.1% Brilliant Blue R-350

(Sigma Aldrich, St. Louis, MO) in 1:1 methanol/water solution for 2 min, de-stained in 1:5:4 ethanol/acetic acid/water solution for 1 min, rinsed briefly with water, and then air-dried for ~1 h prior to imaging.

3.3.7 Real-time quantitative PCR (qPCR)

3.3.7.1 RNA extraction

Total RNA was extracted from approximately 20 mg of muscle tissue using TRIzol® reagent (Ambion Inc., Austin, TX) according to the manufacturer's protocol. Muscle samples were homogenised in 500 μL of TRIzol® reagent using a MagNA Lyser (Roche Diagnostics, Indianapolis, IN) for 20 s at 5500 rpm. After resting for 5 min on ice, 50 μL of 1-bromo-3-chloropropane (BCP) was added and the tube inverted for 30 s, before resting for 10 min at room temperature. The homogenate was centrifuged for 15 min at 13,000 rpm and the upper clear phase collected. Then, 300 μL of isopropanol was added to the tube and inverted briefly to mix, before being stored overnight at -20°C to precipitate the RNA. After overnight incubation, the solution was centrifuged for 60 min at 13,000 rpm to pellet the RNA. The RNA pellet was washed twice by centrifuging in 75% ethanol for 15 min at 13,000 rpm, allowed to air-dry, and then dissolved in 15 μL of nuclease-free water (NFW) (Ambion Inc., Austin, TX). The quantity and quality of RNA was subsequently determined using a spectrophotometer (NanoDrop ND-1000, Thermo Scientific, Wilmington, DE). The purity of RNA was assessed using the ratio between the absorbance at 260 nm and absorbance at 280 nm (mean ± SD; 2.15 ± 0.37), and the ratio between the absorbance at 260 nm and absorbance at 230 nm (1.32 ± 0.51).

3.3.7.2 Reverse transcription

For mRNA analysis, first-strand cDNA was generated from 1 μg RNA in 20 μL reaction buffer using the high-capacity cDNA reverse transcriptase (RT) kit (Applied Biosystems, Australia) according to manufacturer's protocol, with each reaction comprising 2 μL of 10× RT buffer, 0.8 μL of dNTP mix (100 mM), 2 μL of 10× random primers, 1 μL of MultiScribe™ Reverse Transcriptase, 1 μL of RNase inhibitor, 3.2 μL of NFW and 10 μL of RNA sample (diluted to 100 ng/μL). Reverse transcription was then performed with the following conditions: 10 min at 25°C to

anneal primers, 120 min at 37°C for the extension phase, and 5 min at 85°C. Following reverse transcription, samples were DNase-treated (Life Technologies, Carlsbad, CA) and cDNA was stored at -20°C until further analysis.

For miRNA analysis, first-strand cDNA was generated from 50 ng RNA in 15 μL reaction buffer with the TaqMan® miRNA RT kit (#4366596, Applied Biosystems, Australia) and TaqMan® miRNA-specific stem-loop primers (500 ng) (#4427975, Applied Biosystems, Australia) using a modified multiplex protocol (Le Carre et al., 2014). Briefly, miRNA-specific primers were pooled and diluted in NFW to obtain a final dilution of 0.05× each. Then, 6 μL of pooled primer solution was added to the reaction mix containing 0.3 μL of 100 mM dNTP, 3 μL of enzyme (50 U/μL), 1.5 μL of 10× RT buffer, 0.19 μL of RNase inhibitor (20 U/μL) and 50 ng of RNA sample. Reverse transcription was then performed with the following conditions: 30 min at 16°C to anneal primers, 30 min at 42°C for the extension phase, and 5 min at 85°C. The cDNA was then stored at -20°C until further analysis.

3.3.7.3 Real-time quantitative PCR (qPCR)

Real-time PCR was performed using a Realplex^2 Mastercycler PCR system (Eppendorf, Hamburg, Germany) to measure mRNA levels of MuRF-1, Atrogin-1, Fox-O1, myostatin, TSC2, Rheb, PGC-1α, and the reference genes cyclophilin, TBP, GAPDH and β2M. Each PCR reaction was performed in duplicate using a robotic pipetting machine (Epmotion 2100, Eppendorf, Hamburg, Germany) in a final reaction volume of 10 μL containing 5 μL of 2× SYBR green (Bio-Rad Laboratories, Hercules, CA), 0.6 μL of PCR primers (diluted to 15 μM; Sigma Aldrich, St. Louis, MO), 0.4 μL of NFW and 4 μL of cDNA sample (diluted to 5 ng/μL). Conditions for the PCR reactions were: 3 min at 95°C, 40 cycles of 15 s at 95°C/1 min at 60°C, one cycle of 15 s at 95°C/15 s at 60°C, and a ramp for 20 min to 95°C. Each plate was briefly centrifuged before loading into the PCR machine. To compensate for variations in input RNA amounts and efficiency of the reverse transcription, mRNA data were quantified using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) and normalised to the geometric mean (Vandesompele et al., 2002) of the two most stable housekeeping genes analysed (cyclophilin and TBP), determined as previously described (Mane et al., 2008). Details of PCR primers used for all mRNA targets are shown in Table 3.1. Standard and

melting curves were performed for all primers to confirm both primer efficiency and single product amplification, respectively.

A Stratagene MX3000 PCR system (Agilent Technologies, Santa Clara, CA) was used to measure the relative expression levels of miR-1, miR-133a, miR-378, miR-473 and RNU48. Each PCR reaction was performed in triplicate in a final reaction volume of 20 μL containing 7.5 μL of 2× TaqMan® Universal PCR Master Mix, no UNG (#4440040, Applied Biosystems, Australia), 0.5 μL of 1× TaqMan® Small RNA Assay (20×; #4427975, Applied Biosystems, Australia), 0.4 μL of NFW and 4 μL of cDNA sample (diluted to 5 ng/μL). Conditions for the PCR reactions were 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C. Each plate was briefly centrifuged before loading into the PCR machine. To compensate for variations in input RNA amounts and efficiency of the reverse transcription, miRNA data were normalised to RNU48 expression. Details of PCR primers used for all miRNA targets are shown in Table 3.2. Standard curves were performed for all miRNA targets to confirm primer efficiency.

Table 3.1 Details of PCR primers used for Study 1 mRNA analysis

Gene	Forward sequence	Reverse sequence	NCBI reference sequence
MuRF-1	5'-CCTGAGAGCCATTGACTTTGG-3'	5'-CTTCCCTTCTGTGGACTCTTCCT-3'	NM_032588.3
Atrogin-1	5'-GCAGCTGAACAACATTCAGATCAC-3'	5'-CAGCCTCTGCATGATGTTCAGT-3'	NM_058229.3
Fox-O1	5'-TTGTTACATAGTCAGCTTG-3'	5'-TCACTTTCCTGCCCAACCAG-3'	NM_002015.3
Myostatin	5'-CCAGGAGAAGATGGGCTGAA-3'	5'-CAAGACCAAAATCCCTTCTGGAT-3'	NM_005259
TSC2	5'-CCGCAGCATCAGTGTGTC-3'	5'-CACTGGTGAGGGACGTCTG-3'	NM_000548
Rheb	5'-TTTTTGGAATCTTCTGCTAAAGAAA-3'	5'-AAGACTTGCCTTGTGAAGCTG-3'	NM_005614
PGC-1α	5'-GGCAGAAGGCAATTGAAGAG-3'	5'-TCAAAACGGTCCCTCAGTTC-3'	NM_013261.3
TBP	5'-CAGTGACCCAGCAGCATCACT-3'	5'-AGGCCAAGCCCTGAGCGTAA-3'	M55654.1
Cyclophilin	5'-GTCAACCCCACCGTGTTCTTC-3'	5'-TTTCTGCTGTCTTTGGGACCTTG-3'	XM_011508410.1
GAPDH	5'-AAAGCCTGCCGGTGACTAAC-3'	5'-CGCCCAATACGACCAAATCAGA-3'	NM_001256799.2
β2M	5'-TGCTGTCTCCATGTTTGATGTATCT-3'	5'-TCTCTGCTCCCCACCTCTAAGT-3'	NM_004048.2

MuRF-1, muscle RING-finger 1; Fox-O1, forkhead-box O1; TSC2, tuberous sclerosis complex 2; Rheb, ras homologue enriched in brain; PGC-1α, peroxisome proliferator activated receptor gamma co-activator 1 alpha; TBP, TATA binding protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; β2M, beta-2 microglobulin

Table 3.2 Details of PCR primers used for Study 1 microRNA analysis

Target	Assay name	Product ID
miR-1	hsa-miR-1	002222
miR-133a	hsa-miR-133a-3p	002246
miR-378	hsa-miR-378	002243
miR-486	hsa-miR-486-5p	001278
RNU48	RNU48	001006

3.3.8 Muscle glycogen content

Approximately 2-3 mg of freeze-dried muscle tissue was powdered and dissected free of all visible non-muscle tissue. Powdered muscle tissue was then extracted with 250 µL of 2M HCl, incubated at 95°C for 2 h (agitated gently every 20 min), and then neutralised with 750 µL of 0.66M NaOH. Glycogen content was subsequently assayed in triplicate via enzymatic analysis with flourometric detection (Harris et al., 1974). Muscle glycogen values are expressed as $mmol \cdot kg^{-1}$ dry weight.

3.3.9 Statistical analyses

The effect of trial on outcomes was evaluated via a two-way (trial × time) analysis of variance with repeated-measures (RM-ANOVA) (SPSS, Version 21, IBM Corporation, New York, NY). A magnitude-based approach to inferences using the standardised difference (effect size, ES), was also used as previously described (Hopkins et al., 2009). The magnitude of effects were defined according to thresholds suggested by Hopkins (Hopkins et al., 2009), whereby <0.2 = trivial, 0.2-0.6 = small, 0.6-1.2 = moderate, 1.2-2.0 = large, 2.0-4.0 = very large and >4.0 = extremely large effects. Lacking information on the smallest meaningful effect for changes in protein phosphorylation and gene expression, the threshold for the smallest worthwhile effect was defined as an ES of 0.4, rather than the conventional threshold of 0.2. Magnitude-based inferences about effects were made by qualifying the effects with probabilities that reflected the uncertainty in the magnitude of the true effect (Batterham & Hopkins, 2005); 25-75%, ‘possibly’; 75-95%, ‘likely’; 95-99.5%, ‘very likely’; >99.5%, ‘most likely’. Effects deemed at least 75% ‘likely’ to be substantial (according to the overlap between the uncertainty in the magnitude of the true effect and the smallest worthwhile

change (Batterham & Hopkins, 2005)) were reported. Exact *P* values were also determined for each comparison, derived from paired (for within-trial comparisons) or unpaired (for between-trial comparisons) *t*-tests, with a Bonferroni correction applied to correct for multiple comparisons (SPSS, Version 21, IBM Corporation, New York, NY). Physiological (blood lactate, blood glucose, heart rate) and psychological (rating of perceived exertion [RPE]) responses to exercise are reported as mean values ± SD, whereas molecular (protein signalling, mRNA and microRNA expression) and muscle glycogen data were log-transformed before analysis to reduce non-uniformity of error (Hopkins et al., 2009) and reported as mean between-condition percentage differences ±90 % confidence limit (CL).

3.4 Results[1] [2]

3.4.1 Physiological and psychological responses to exercise

3.4.1.1 Heart rate and rating of perceived exertion (RPE) responses

Both heart rate (mean ± SD, 171 ± 9 and 135 ± 12 beats·min^{-1}, respectively) and rating of perceived exertion (RPE) during cycling (16 ± 2 and 12 ± 2 AU, respectively) were higher during HIT compared with MICT ($P < 0.05$; Table 3.3).

3.4.1.2 Venous blood lactate and glucose responses

Venous blood lactate levels were higher for HIT compared with MICT at all time points during cycling and recovery from cycling ($P < 0.05$; Table 3.3). Blood lactate was also higher for HIT+RE at 10, 30 and 60 min post-RE compared with RE (Table 3.4; $P < 0.05$). Venous blood glucose was higher for HIT compared with MICT only after 34 min of cycling, and at 2, 5 and 10 min of recovery from cycling ($P < 0.05$; Table 3.3).

[1] All raw data for this Chapter is available in Appendix K.
[2] Extended within- and between-trial comparison data for this Chapter are presented in Appendices M and N, respectively.

Table 3.3 Physiological and psychological (RPE) responses to HIT and work-matched MICT protocols for Study 1.

	Time (min)										
	Rest	Pre	10	16	22	28	34	+2	+5	+10	+15
Lactate (mmol·L^{-1})											
HIT	0.6 ± 0.2	0.8 ± 0.2	2.8 ± 1.0 #	4.3 ± 1.5 *#	5.3 ± 1.7 *#	5.7 ± 1.9 *#	6.1 ± 2.0 *#	5.8 ± 1.8 *#	5.6 ± 2.2 #	4.9 ± 2.2 #	4.1 ± 2.0 #
MICT	0.6 ± 0.2	0.9 ± 0.2	1.6 ± 0.4 #	2.1 ± 0.6	2.3 ± 0.7	2.4 ± 0.6	2.4 ± 0.7	2.2 ± 0.6	2.0 ± 0.6	1.6 ± 0.4	1.4 ± 0.2
Glucose (mmol·L^{-1})											
HIT	4.4 ± 0.7	4.3 ± 1.9	3.4 ± 1.2	3.7 ± 0.7	4.1 ± 0.1	4.3 ± 0.5	4.6 ± 0.5 #	5.0 ± 0.8 #	4.8 ± 0.5 #	4.6 ± 0.6 #	4.3 ± 0.5
MICT	4.4 ± 0.3	4.6 ± 1.0	3.6 ± 0.6	3.4 ± 0.3	3.5 ± 0.4	3.7 ± 0.4	3.8 ± 0.6	4.0 ± 0.4	4.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.5
Heart rate (beats·min^{-1})											
HIT	-	63 ± 12	160 ± 9 *#	168 ± 7 *#	173 ± 9 *#	176 ± 9 *#	178 ± 8 *#	-	-	-	-
MICT	-	62 ± 10	123 ± 9 *	133 ± 12 *	139 ± 13 *	141 ± 12 *	141 ± 12 *	-	-	-	-
RPE (AU)											
HIT	-	6 ± 0	14 ± 2 *#	15 ± 2 *#	16 ± 2 *#	17 ± 2 *#	18 ± 1 *#	-	-	-	-
MICT	-	6 ± 0	10 ± 1 *	11 ± 2 *	12 ± 2 *	12 ± 2 *	13 ± 2 *	-	-	-	-

Values are means ± SD. HIT, = high-intensity interval training cycling; MICT, continuous cycling; RPE, rating of perceived exertion. *, $P < 0.05$ vs. rest; #, $P < 0.05$ vs. MICT at same time point.

Table 3.4 Venous blood lactate and glucose responses following resistance exercise performed alone (RE) and when performed after either HIT (HIT+RE) or work-matched MICT (MICT+RE) for Study 1.

	Time (min)							
	End	+2	+5	+10	+30	+60	+120	+180
Lactate ($mmol \cdot L^{-1}$)								
RE	1.7 ± 0.7	1.7 ± 0.8	1.5 ± 0.6	1.2 ± 0.4	0.8 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2
HIT+RE	2.2 ± 0.9	2.3 ± 0.9	2.1 ± 0.8	1.7 ± 0.7 ‡	1.1 ± 0.4 ‡	0.9 ± 0.4 ‡	0.7 ± 0.4	0.6 ± 0.1
MICT+RE	1.7 ± 1.7	1.7 ± 0.7	1.5 ± 0.3	1.4 ± 0.4	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
Glucose ($mmol \cdot L^{-1}$)								
RE	4.4 ± 0.3	4.1 ± 0.6	4.4 ± 0.2	4.3 ± 0.3	4.3 ± 0.3	4.4 ± 0.6	4.4 ± 0.3	4.5 ± 0.2
HIT+RE	4.1 ± 0.4	4.2 ± 0.3	4.2 ± 0.2	4.1 ± 0.4	4.2 ± 0.3	4.2 ± 0.3	4.3 ± 0.3	4.3 ± 0.3
MICT+RE	4.3 ± 0.3	4.3 ± 0.3	4.2 ± 0.4	4.2 ± 0.4	4.4 ± 0.2	4.3 ± 0.2	4.3 ± 0.3	4.4 ± 0.2

Values are means ± SD. RE, resistance exercise; HIT+RE, = high-intensity interval training cycling and resistance exercise; MICT+RE, continuous cycling and resistance exercise. *, $P < 0.05$ vs. rest; ‡, $P < 0.05$ vs. RE at same time point.

3.4.2 Muscle glycogen content

There was a main effect of time for changes in muscle glycogen content (P < 0.001). Muscle glycogen content was unchanged across time following RE (Figure 3.2); however, it was reduced at POST for both HIT+RE (mean difference ±90% CL, 50 ±23%; ES ±90% CL, -2.91 ±1.86; P = 0.022) and MICT+RE (40 ±11%; ES, -2.10 ±0.76; P = 0.004) compared with PRE. Post-RE, muscle glycogen content was also lower at for HIT+RE and MICT+RE at +1 h (62 ±15%; ES, -4.01 ±1.56; P = 0.002 and 43 ±19%; ES, -2.36 ±1.38; P = 0.014, respectively) and +3 h (45 ±11%; ES, -2.46 ±0.80; P = 0.001 and 31 ±18%; ES, -1.55 ±1.07; P = 0.033, respectively) compared with RE. Muscle glycogen content was also lower for HIT+RE compared with MICT+RE at +1 h (33 ±22%; ES, -1.65 ±1.32; P = 0.034).

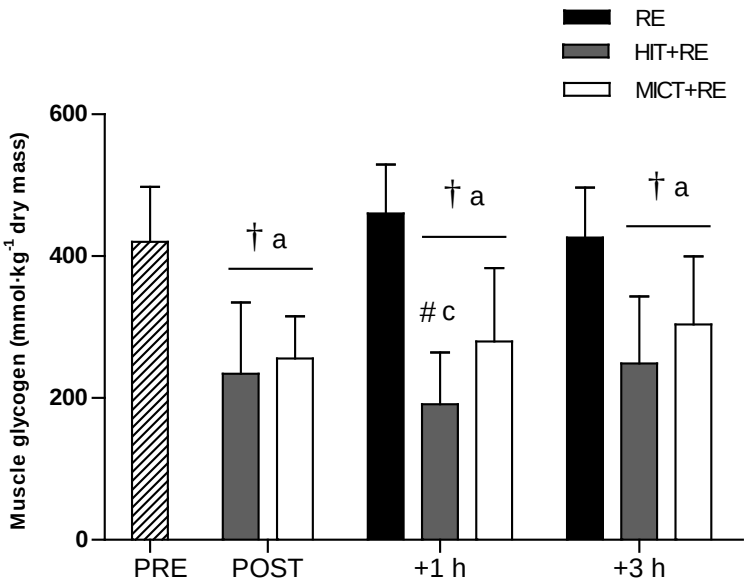

Figure 3.2 Muscle glycogen content of the vastus lateralis before (PRE), 1 h (+1 h) and 3 h (+3 h) after a bout of resistance exercise (RE), and when RE was preceded (POST) by either high-intensity interval training (HIT; HIT+RE) or moderate-intensity continuous training (MICT; MICT+RE). Data shown are means ± SD. † = P < 0.05 vs. RE at same time point, # = P < 0.05 vs. MICT+RE at same time point. Substantially greater at same time point vs. a = RT, c = MICT+RT.

3.4.3 Signalling responses

p-AMPK^{Thr172}. Following RE, the phosphorylation of AMPK^{Thr172} was increased compared with PRE at +1 h (137 ±162%; ES, 1.12 ±0.83; P = 0.033) and +3 h (201 ±143%; ES, 1.43 ±0.60; P = 0.002; Figure 3.3A). Compared with PRE, there were

likely moderate effects for elevated AMPK phosphorylation at POST for HIT+RE (107 ±163%; ES, 0.94 ±0.94; *P* = 0.144) and MICT+RE (118 ±160%; ES, 1.01 ±0.88; *P* = 0.074). A likely moderate effect was also seen for higher AMPK phosphorylation at +1 h for MICT+RE compared with RE (59 ±61%; ES, 0.61 ±0.48; *P* = 0.085). There were no differences in AMPK phosphorylation between HIT+RE and MICT+RE at any time point.

p-ACC^{Ser79}. There were main effects of time (*P* < 0.001), trial (*P* = 0.002), and an interaction effect (*P* < 0.001), for ACC^{Ser79} phosphorylation. Compared with PRE, the phosphorylation of ACC^{Ser79} was lower for RE at +1 h (60 ±24%; ES, -1.15 ±0.70; *P* = 0.017; Figure 3.3B) and +3 h (82 ±28%; ES, -2.15 ±1.53; *P* = 0.040). ACC phosphorylation was higher at POST compared with PRE for both HIT+RE (530 ±145%; ES, 2.29 ±0.28; *P* <0.001) and MICT+RE (451 ±274%; ES, 2.13 ±0.60; *P* = 0.002). Post-RE, ACC phosphorylation was higher for HIT+RE compared with RE both +1 h (133 ±96%; ES, 1.06 ±0.50; *P* = 0.038) and +3 h (458 ±215%; ES, 2.14 ±0.47; *P* = 0.043). Moreover, ACC phosphorylation was higher for HIT+RE compared with MICT+RE at +1 h (161 ±218%; ES, 1.19 ±0.95; *P* = 0.046) and +3 h (161 ±121%; ES, 1.20 ±0.56; *P* = 0.005).

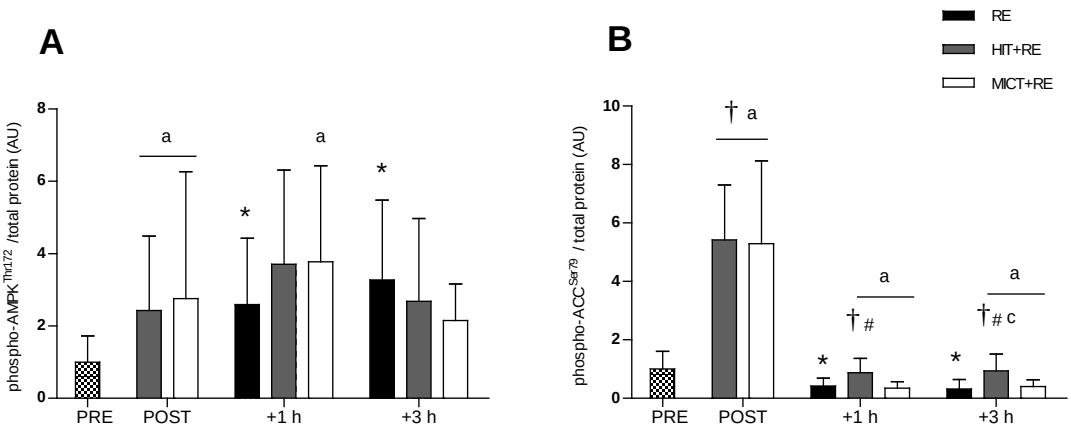

Figure 3.3 Phosphorylation of AMPK^{Thr172} (A) and ACC^{Ser79} (B) before (PRE), 1 h and 3 h after a bout of resistance exercise (RE), and when RE was preceded (POST) by either high-intensity interval training (HIT; HIT+RE) or moderate-intensity continuous training (MICT; MICT+RE). Data shown are means ± SD and expressed relative to the PRE value for the RE trial. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. RE at same time point, # = *P* < 0.05 vs. MICT+RE at same time point. Substantially greater at same time point vs. a = RT, c = MICT+RT.

p-mTOR^{Ser2448}. There was a main effect of trial for mTOR^{Ser2448} phosphorylation (P = 0.035). The phosphorylation of mTOR^{Ser2448} was unchanged over time by RE; however, it was higher at POST for HIT+RE compared with both PRE (105 ±34%; ES, 0.84 ±0.19; P = 0.011; Figure 3.4A) and MICT+RE (44 ±21%; ES, 0.67 ±0.44; P = 0.014). At +1 h, mTOR phosphorylation was higher for HIT+RE compared with MICT+RE (128 ±67%; ES, 0.83 ±0.29; P = 0.003). mTOR phosphorylation was also likely moderately higher at +3 h for HIT+RE compared with both RE (91 ±84%; ES, 0.75 ±0.49; P = 0.148) and MICT+RE (138 ±285%; ES, 1.01 ±1.18; P = 0.150).

p-p70S6K^{Thr389}. There was a main effect of time for p70S6K^{Thr389} phosphorylation (P = 0.001). RE increased p70S6K^{Thr389} phosphorylation above PRE values at +1 h (171 ±95%; ES, 1.39 ±0.48; P = 0.002; Figure 3.4B) and +3 h (65 ±56%; ES, 0.70 ±0.47; P = 0.025). At POST, likely moderate elevations in p70S6K phosphorylation were noted for HIT+RE (77 ±55%; ES, 0.80 ±0.43; P = 0.154) and MICT+RE (60 ±67%; ES, 0.66 ±0.57; P = 0.178) compared with PRE. There were no differences in p70S6K phosphorylation between HIT+RE and MICT+RE at any time point.

p-rps6$^{Ser235/236}$. There was a main effect of time for changes in rps6$^{Ser235/236}$ phosphorylation (P = 0.030). RE increased rps6$^{Ser235/236}$ phosphorylation above PRE values at +1 h (215 ±155%; ES, 1.21 ±0.50; P = 0.029; Figure 3.4C) and + 3 h (203 ±226%; ES, 1.17 ±0.73; P = 0.018). At POST, rps6 phosphorylation was higher for HIT+RE compared with PRE (153 ±116%; ES, 0.98 ±0.47; P = 0.023). There were no differences in rps6 phosphorylation between HIT+RE and MICT+RE at any time point.

p-eEF2^{Thr56}. The phosphorylation status of eEF2^{Thr56} was unchanged over time by RE (Figure 3.4D). However, likely large reductions in eEF2 phosphorylation were noted at POST for both HIT+RE (-34 ±33%; ES, -1.72 ±1.98; P = 0.144) and MICT+RE (-37 ±23%; ES, -1.87 ±1.47; P = 0.035) compared with PRE. Moreover, compared with RE, likely moderate decreases in eEF2 phosphorylation were noted at +1 h for both HIT+RE (-16 ±10%; ES, -0.71 ±0.49; P = 0.050) and MICT+RE (-21 ±24%; ES, -0.99 ±1.22; P = 0.179). There were no differences in eEF2 phosphorylation between HIT+RE and MICT+RE at any time point.

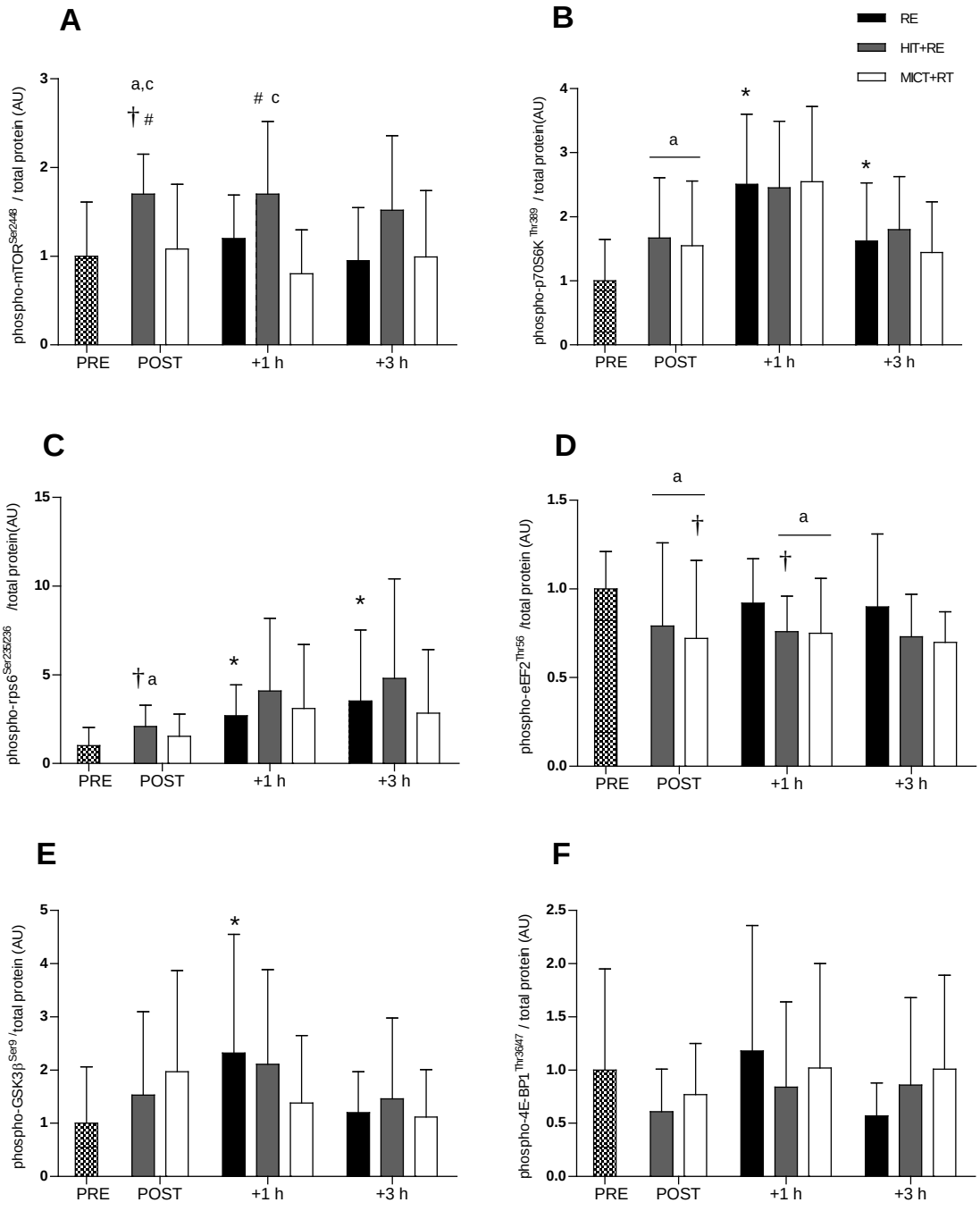

Figure 3.4 Phosphorylation of mTOR^{Ser2448} (A), p70S6K^{Thr389} (B), rps6$^{Ser235/236}$ (C), eEF2^{Thr56} (D), GSK-3β$^{Ser/9}$ (E) and 4E-BP1$^{Thr36/47}$ (F) before (PRE), 1 h and 3 h after a bout of resistance exercise (RE), and when RE was preceded (POST) by either high-intensity interval training (HIT; HIT+RE) or moderate-intensity continuous training (MICT; MICT+RE). Data shown are means ± SD and expressed relative to the PRE value for the RE trial. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. RE at same time point, # = *P* < 0.05 vs. MICT+RE at same time point. Substantially greater at same time point vs. a = RT, c = MICT+RT.

p-GSK-3β^{Ser9}. There was a main effect of time for GSK-3β^{Ser9} phosphorylation (P = 0.021). RE increased GSK-3β^{Ser9} phosphorylation above PRE values at + 1 h (130 ±87%; ES, 0.70 ±0.31; P = 0.002; Figure 3.4E), returning to baseline at +3 h. There were no between-trial differences in GSK-3β phosphorylation at any time point.

p-4E-BP1$^{Thr36/47}$. The phosphorylation status of 4E-BP1$^{Thr36/47}$ was unchanged across time by RE and was not different between trials at any time point (Figure 3.4F).

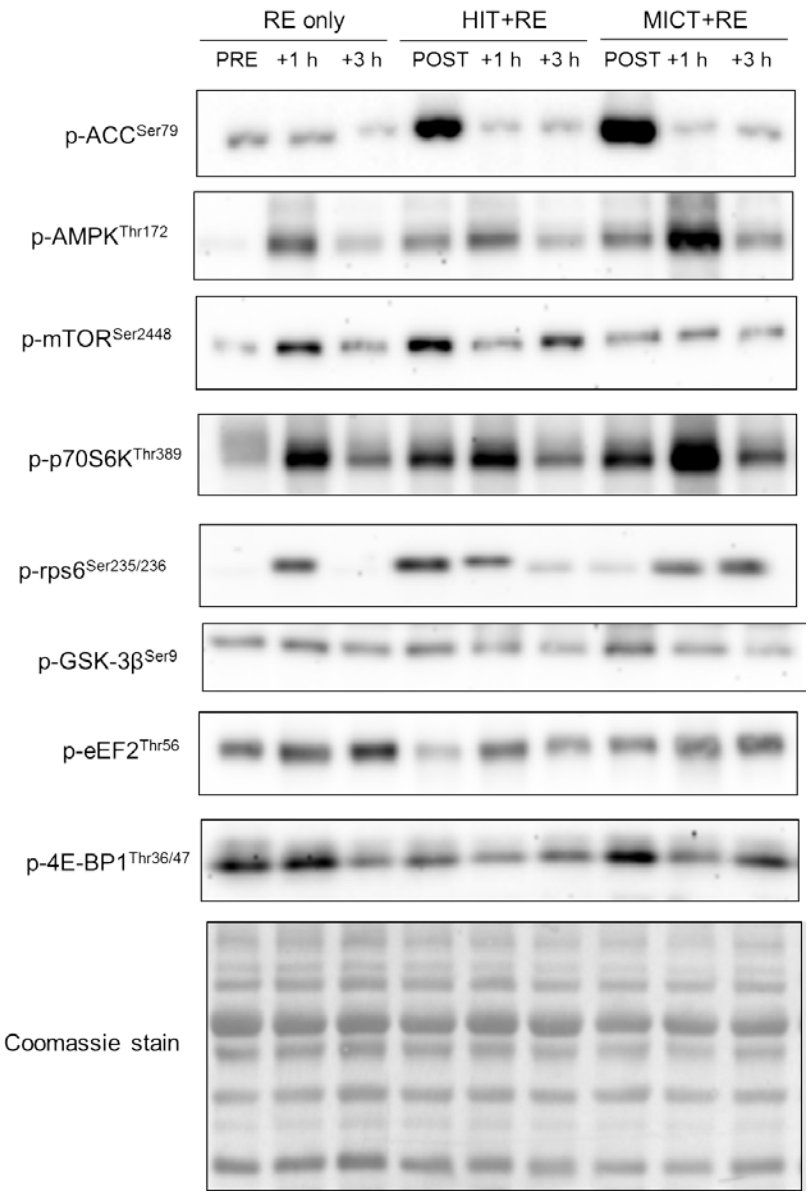

Figure 3.5 Representative western blots for the phosphorylation (p-) of signalling proteins before (PRE), 1 h and 3 h after a bout of resistance exercise (RE), and when RE was preceded by either high-intensity interval training (HIT; HIT+RE) or moderate-intensity continuous training (MICT; MICT+RE).

3.4.4 mRNA responses

MuRF-1 mRNA. There was a main effect of time for MuRF-1 mRNA expression (P = 0.048). MuRF-1 expression was not altered by RE at +3 h compared with PRE (Figure 3.6A); however, there were likely small elevations in MuRF-1 expression at +3 h for both HIT+RE (585 ±684 %; ES, 0.52 ±0.64; P = 0.170) and MICT+RE (535 ±464%; ES, 0.33 ±0.20; P = 0.016) compared with RE. There were no differences in MuRF-1 expression between HIT+RE and MICT+RE at any time point.

Atrogin-1 mRNA. Atrogin-1 mRNA expression was not altered by RE at +3 h compared with PRE (Figure 3.6B), and was not different between trials at any time point.

Fox-O1 mRNA. Fox-O1 mRNA expression was unaltered by RE at +3 h compared with PRE (Figure 3.6C). There was a possibly small increase in Fox-O1 mRNA expression at +3 h for HIT+RT compared with MICT+RT (62 ±58 %; ES, 0.34 ±0.25; P = 0.036).

Myostatin mRNA. Myostatin mRNA expression was unaltered by RE at +3 h compared with PRE (Figure 3.6D). At POST, myostatin expression was greater for HIT+RE compared with MICT+RE (53 ±18 %; ES, 0.60 ±0.30; P = 0.044).

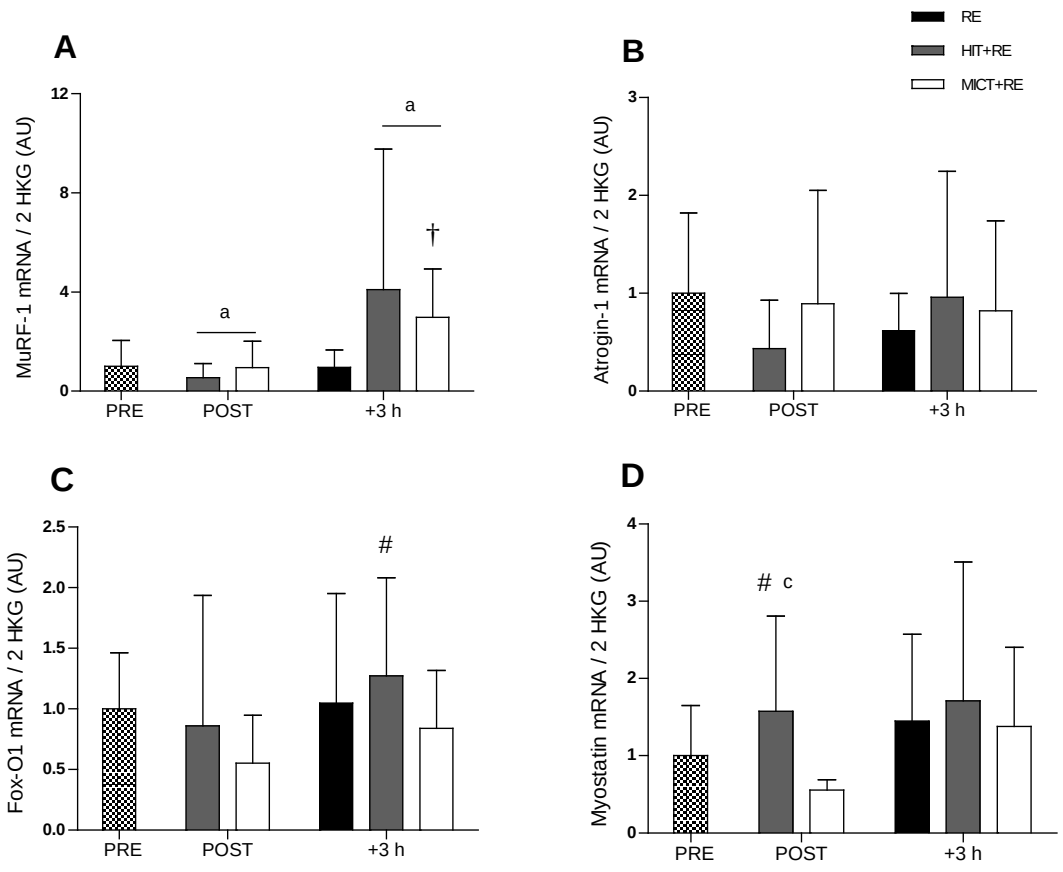

Figure 3.6 mRNA expression of MuRF-1 (A), Atrogin-1 (B), Fox-O1 (3C) and myostatin (D) relative to cyclophillin and TBP expression before (PRE), 1 h and 3 h after a bout of resistance exercise (RE), and when RE was preceded (POST) by either high-intensity interval training (HIT; HIT+RE) or moderate-intensity continuous training (MICT; MICT+RE). Data shown are means ± SD and expressed relative to the PRE value for the RE trial. † = *P* < 0.05 vs. RE at same time point, # = *P* < 0.05 vs. MICT+RE at same time point. Substantially greater at same time point vs. a = RT, c = MICT+RT.

PGC-1α mRNA. There was a main effect of time for PGC-1α mRNA expression (P = 0.016). PGC-1α expression was unchanged after RE (Figure 3.7A); however, there were likely small increases in PGC-1α expression at +3 h for both HIT+RE (788 ±878%; ES, 0.54 ±0.54; P = 0.098) and MICT+RE (604 ±403%; ES, 0.59 ±0.42; P = 0.033) compared with RE. There were no differences in PGC-1α expression between HIT+RE and MICT+RE at any time point.

Rheb/TSC2 mRNA. mRNA expression of Rheb or TSC2 was not altered by RE, and was not different between trials at any time point (Figures 3.7B and 3.7C).

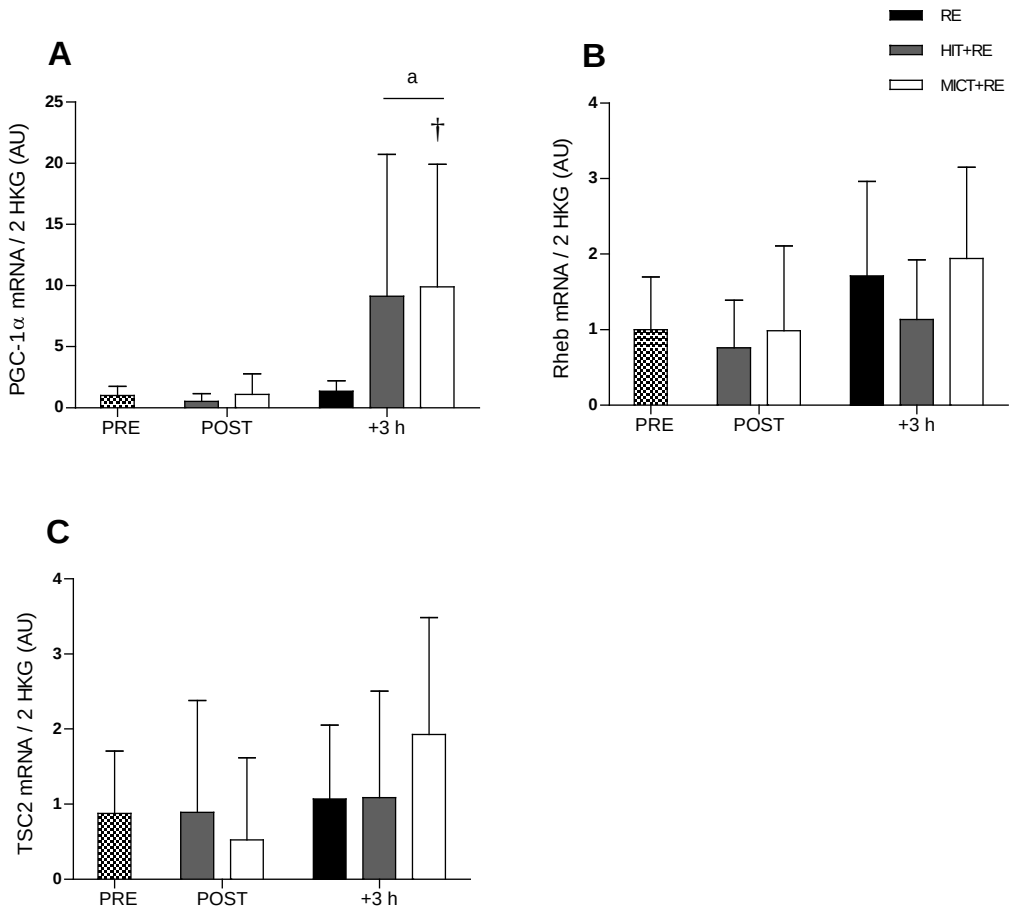

Figure 3.7 mRNA expression of PGC-1α (A), Rheb (B) and TSC2 (C) relative to cyclophillin and TBP expression before (PRE), 1 h and 3 h after a bout of resistance exercise (RE), and when RE was preceded (POST) by either high-intensity interval training (HIT; HIT+RE) or moderate-intensity continuous training (MICT; MICT+RE). Data shown are means ± SD and expressed relative to the PRE value for the RE trial. † = *P* < 0.05 vs. RE at same time point, # = *P* < 0.05 vs. MICT+RE at same time point. Substantially greater at same time point vs. a = RT.

3.4.5 microRNA responses

miR-1. There was a main effect of time for miR-1 expression (P = 0.043). However, miR-1 expression was not altered by RE, and was not substantially different between trials at any time point (Figure 3.8A).

miR-133a. There was a main effect of time for miR-133a expression (P = 0.035). RE decreased miR-133a expression relative to PRE at +3 h (27 ±14%; ES, -0.38

±0.23; *P* = 0.026; Figure 3.8B). For the HIT+RE trial, miR-133a expression was lower at POST compared with PRE (-40 ±13%; ES, -0.63 ±0.27; *P* = 0.014), and there were likely small-to-moderate reductions in miR-133a expression at +1 h compared with both RE (-35 ±28%; ES, -0.53 ±0.50; *P* = 0.068) and MICT+RE (-43 ±30%; ES, -0.68 ±0.61; *P* = 0.053). There were no between-condition differences in miR-133a expression at +3 h.

miR-378. RE did not alter miR-378 expression relative to PRE (Figure 3.8C). However, there were likely moderate reductions in miR-378 expression at +1 h for HIT+RE compared with both RE (-37 ±26%; ES, -0.79 ±0.70; *P* = 0.052) and MICT+RE (-42 ±28%; ES, -0.94 ±0.81; *P* = 0.046). There were no between-condition differences in miR-378 expression at +3 h.

miR-486. There was a main effect of trial for miR-486 expression (*P* = 0.048). RE did not alter miR-486 expression relative to PRE (Figure 3.8D). However, miR-486 expression was reduced at +1 h for HIT+RE compared with both RE (-36 ±17%; ES, -1.02 ±0.59; *P* = 0.040) and MICT+RE (38 ±15%; ES, -1.10 ±0.56; *P* = 0.015). There were no between-condition differences in miR-486 expression at +3 h.

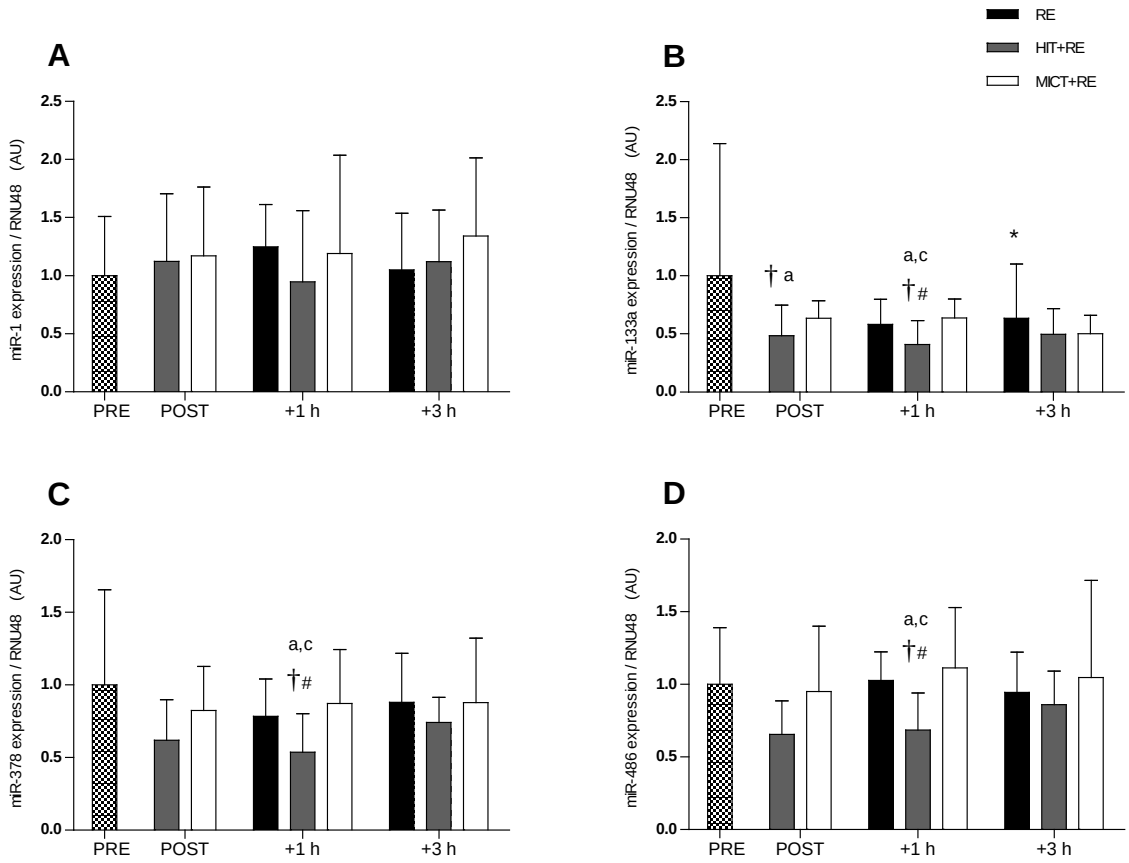

Figure 3.8 Expression levels of miR-1 (A), miR-133a (B), miR-378 (C) and miR-486 (D) relative to RNU48 expression immediately before (PRE), +1 h and +3 h following resistance exercise (RE), and when RE was preceded (POST) by either HIT (HIT+RE) or moderate-intensity continuous training (MICT+RE). Data shown are means ± SD and expressed relative to the PRE value for the RE trial. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. RE at same time point, # = *P* < 0.05 vs. MICT+RE at same time point. Substantially greater at same time point vs. a = RT, c = MICT+RT.

3.5 Discussion

The aim of this study was to compare the effects of concurrent exercise incorporating either HIT or MICT on anabolic and catabolic molecular responses in skeletal muscle, compared with RE performed alone. The major findings were that i) prior HIT and MICT induced similar muscle glycogen depletion and increased ACC phosphorylation prior to commencing a subsequent RE bout; ii) despite these residual effects, mTORC1 signalling was generally not compromised during early recovery from concurrent exercise compared with RE alone; iii) concurrent exercise incorporating HIT

and MICT similarly increased MuRF-1 mRNA expression compared with RE alone; and iv) prior HIT, but not MICT, reduced the expression of miRNAs implicated in exercise-induced skeletal muscle adaptations after subsequent RE, compared with RE performed alone.

Interference to mTORC1 signalling has previously been investigated as a mechanism contributing to attenuated RE adaptations during concurrent training in humans (Apro et al., 2015; Apro et al., 2013; Donges et al., 2012; Lundberg et al., 2012; Pugh et al., 2015). However, whether higher endurance exercise intensities, previously shown to further increase AMPK activity in skeletal muscle compared with lower-intensity endurance exercise (Rose et al., 2009b), are associated with greater interference to mTORC1 signalling following concurrent exercise has not been examined. In the present study, the RE-induced phosphorylation responses of several mTORC1 pathway intermediates were generally not compromised when RE was performed after either HIT or MICT. For example, the phosphorylation of p70S6K, which reportedly correlates with load-induced hypertrophy in both rodent (Baar & Esser, 1999) and human (Terzis et al., 2008) models, was similar between conditions at 1 h post-RE. Few differences in post-exercise signalling responses were observed between concurrent exercise and single-mode RE, although eEF2 phosphorylation was lower at +1 h for HIT+RE compared with RE alone, and mTOR phosphorylation was lower at +1 h for MICT+RE compared with HIT+RE and RE alone. These observations align with others showing that mTORC1 signalling and rates of MPS following a single bout of concurrent exercise are not attenuated compared with RE performed alone (Apro et al., 2013; Donges et al., 2012; Lundberg et al., 2012). For example, performing 30 min of cycling at 70% $\dot{V}O_{2max}$ 15 min after RE does not compromise the post-exercise phosphorylation of mTOR, p70S6K and eEF2 compared with RE performed alone (Apro et al., 2013). Moreover, rates of MPS following RE are not compromised by the addition of either 20 min of MICT cycling at 55% peak aerobic power (Donges et al., 2012) or 90 min at 60% peak aerobic power (Carrithers et al., 2007). These data add support to the notion that concurrent exercise appears not to interfere with mTORC1 signalling compared with single-mode RE, at least within the early post-exercise recovery period (<3 h).

Rather than inhibit anabolic responses, the endurance exercise protocols in the present study were themselves clearly sufficient to promote mTORC1 signalling. The HIT protocol was particularly effective for increasing mTOR and rps6 phosphorylation

compared with MICT, whereas both protocols similarly increased p70S6K phosphorylation at POST. These data align with evidence that endurance exercise is sufficient to induce anabolic responses in skeletal muscle, including increased mTORC1 signalling and rates of MPS, at least in relatively training-unaccustomed individuals (Di Donato et al., 2014; Mascher et al., 2011). For example, mTOR/p70S6K phosphorylation and rates of mixed MPS are increased following 1 h of cycling at 65-70% $\dot{V}O_{2max}$ (Mascher et al., 2011), and high- (60% W_{max} for 30 min), but not low- (30% W_{max} for 60 min) intensity continuous cycling induces sustained (24-28 h post-exercise) increases in myofibrillar MPS (Di Donato et al., 2014). The importance of training status in dictating the specificity of early post-exercise responses must also be considered when interpreting these data. For example, mTOR phosphorylation is preferentially increased following RE, but not endurance exercise, in training-accustomed individuals (Vissing et al., 2011). Similarly, both myofibrillar and mitochondrial MPS are similarly increased by resistance and endurance exercise in a training-unaccustomed state, whereas RE preferentially stimulates increased myofibrillar MPS after training (Wilkinson et al., 2008). It therefore appears likely that concurrent exercise modulates early post-exercise molecular responses over time, potentially progressing towards an attenuation of anabolic responses with concurrent exercise compared with single-mode RE. Indeed, the greater increase in p70S6K phosphorylation observed after a single bout of concurrent exercise compared with single-mode RE is abolished after five weeks of training (Fernandez-Gonzalo et al., 2013). The participants in the present study were not accustomed to the specific exercise protocols employed during the experimental trials, which may have influenced the ability of endurance exercise to modulate anabolic responses in skeletal muscle. Further work is required to determine the influence of training status and the potential progression of interference to anabolic responses and adaptations in human skeletal muscle with concurrent training.

In agreement with others (Coffey et al., 2009a; Coffey et al., 2009b; Lundberg et al., 2012; Pugh et al., 2015) the RE protocol employed in the present study was insufficient to modulate $mTOR^{Ser2448}$ phosphorylation in the early post-exercise recovery period, It is possible the lack of altered $mTOR^{Ser2448}$ phosphorylation may have been due to a lack of metabolic stress induced in skeletal muscle by the RE protocol, and/or that RE sets were not taken to the point of concentric failure. Resistance exercise sets were low in volume (i.e., 5 repetitions per set) and not taken to failure in the present

study to maximise the divergence between the endurance and RE stimuli (i.e., low-force, high metabolic stress vs. high-force, low metabolic stress). Despite the lack of altered $mTOR^{Ser2448}$ phosphorylation, the RE protocol in the present study was sufficient to induce signalling downstream of mTORC1, including increased p70S6K and rps6 phosphorylation. There is evidence that downstream targets of mTORC1 (e.g., p70S6K and 4E-BP1) may be activated in skeletal muscle in the absence of mTORC1 activation (Liu et al., 2013). This observation is in agreement with the present data and suggests these targets may have been activated via mTORC1-independent mechanisms.

Muscle glycogen depletion, a modulator of AMPK activity (Yeo et al., 2010), has been suggested as a potential mechanism by which endurance exercise may attenuate anabolic responses in skeletal muscle following RE. While early work (Creer et al., 2005) suggested Akt phosphorylation was attenuated in human skeletal muscle when RE was performed in a glycogen-depleted state, more recent evidence suggests that muscle glycogen availability does not modulate mTORC1 signalling or rates of MPS following RE (Camera et al., 2012). The HIT and MICT protocols in the present study induced similar muscle glycogen depletion, so that glycogen content was comparably reduced at POST for the HIT+RE and MICT+RE trials compared with PRE. Although it was initially hypothesised HIT would induce greater glycogen depletion, based on intensity-dependent glycogen depletion during continuous exercise (Gollnick et al., 1974), the present findings align with previous work showing similar muscle glycogen depletion, at least at the whole-muscle level, following constant or variable-intensity cycling (Suriano et al., 2010) and running (Bartlett et al., 2012) matched for total work. Mirroring these responses were the comparable increases in ACC phosphorylation at POST with prior HIT or MICT, which may suggest similar increases in AMPK activity (Park et al., 2002). This is in agreement with Bartlett et al. (Bartlett et al., 2012) who observed similarly increased AMPK phosphorylation following work-matched HIT and MICT running. Although HIT and MICT induced similar glycogen depletion at POST, muscle glycogen content was ~33% lower at +1 h for HIT+RE compared with MICT+RE. A similar pattern was noted for ACC phosphorylation, which was similarly elevated at +1 h and +3 h (~161%) for HIT+RE compared with MICT+RE. Nevertheless, the present data supports the notion that muscle glycogen availability, at least in the ranges reported here, does not modulate exercise-induced mTORC1 signalling in human skeletal muscle and does not appear to mediate any interference effect within the time course examined.

In agreement with some (Coffey et al., 2009a; Dreyer et al., 2006; Koopman et al., 2006), but not all (Apro et al., 2013; Camera et al., 2010) studies, increased AMPK phosphorylation was observed following RE. Potentially, the training status of the participants in the present study may have influenced these results, as AMPK phosphorylation has been shown to be increased by RE in participants un-accustomed to the specific exercise protocol (Coffey et al., 2006b; Dreyer et al., 2006), yet AMPK activity is unchanged after RE in resistance-trained subjects (Apro et al., 2015). Despite the increased AMPK phosphorylation induced by RE, this was not associated with increases in ACC phosphorylation, a downstream target of AMPK and a marker of its activity (Ha et al., 1994; Park et al., 2002), which instead decreased over time for the RE trial. There appears, therefore, to be an apparent disconnect between the AMPK and ACC phosphorylation responses for the RE trial, for which there appears to be no clear explanation. Dissociations between AMPK and ACC phosphorylation have been reported in human skeletal muscle with increases in exercise intensity (Chen et al., 2003) and during prolonged, submaximal exercise (Wojtaszewski et al., 2002). Nevertheless, given that ACC phosphorylation may provide a better indication of AMPK activity compared with AMPK phosphorylation (Park et al., 2002), we have primarily used the ACC phosphorylation responses when interpreting the effects of the exercise protocols on AMPK activity.

In addition to altering post-exercise responses governing MPS, another mechanism by which concurrent exercise might mediate the interference effect is by exacerbating rates of protein degradation (Apro et al., 2015). MuRF-1 and Atrogin-1 are E3 ubiquitin ligases implicated in the tagging and subsequent degradation of contractile proteins via the ubiquitin proteasome system (Bodine et al., 2001a). In the present study, RE had little effect on either MuRF-1 or Atrogin-1 gene expression relative to baseline. However, relative to RE alone, MuRF-1 mRNA abundance was similarly increased at +3 h when RE was performed after HIT or MICT. While data regarding ubiquitin ligase expression after concurrent exercise incorporating divergent endurance training intensities is scarce, increased MuRF-1 mRNA expression has been shown in separate studies when RE is preceded by either HIT (Apro et al., 2015; Pugh et al., 2015) or MICT cycling (Lundberg et al., 2014b). The present data lend support to the notion that concurrent exercise increases the expression of markers of protein degradation compared with single-mode RE, and extends current knowledge by suggesting this response is unrelated to the endurance exercise intensity employed. The

significance of increased MuRF-1 expression following concurrent exercise is unclear, however, given that increased post-exercise ubiquitin ligase activity may be necessary to facilitate skeletal muscle remodelling by removing damaged proteins and/or providing amino acid substrates for incorporation into newly synthesised proteins (Sanchez et al., 2014b).

Additional molecular mechanisms implicated in the regulation of exercise-induced skeletal muscle adaptations were also investigated. miRNAs have been associated with the regulation of skeletal muscle mass (Hitachi & Tsuchida, 2013), and their expression levels are altered following both single bouts of exercise (Drummond et al., 2008c; Russell et al., 2013; Zacharewicz et al., 2014) and short-term exercise training (Davidsen et al., 2011; Russell et al., 2013). However, no studies have investigated whether miRNA expression is altered following concurrent compared with single-mode RE. The myomiRs miR-1 and miR-133a can target members of the IGF-1/Akt pathway *in vitro*, including IGF-1 (Elia et al., 2009), the IGF-1 receptor (Elia et al., 2009) as well as HSP70 (Kukreti et al., 2013). This suggests they may play a role in regulating muscle hypertrophy, although this requires experimental validation *in vivo*. Expression of both miR-1 and miR-133a is reduced during functional overload in mice (McCarthy & Esser, 2007b), and miR-1 expression is reduced 1 h after a single bout of RE with amino acid ingestion (Drummond et al., 2008c), whereas a single bout of endurance exercise increases miR-1 expression (Russell et al., 2013). Nonetheless, contrary to previous observations (Drummond et al., 2008c), RE was insufficient to alter miR-1 expression and there were no differences in this response following either HIT+RE or MICT+RE. When performed alone, RE decreased miR-133a expression at +3 h compared with baseline, while prior HIT further reduced miR-133a expression at PRE and at +1 h compared with RE. These observations suggest that concurrent HIT+RE may promote a more favourable post-exercise anabolic response compared with RE alone by reducing miR-133a expression, thereby alleviating any potential miR-133a-mediated repression of members of the IGF-1/Akt signalling pathway.

Increased expression of miR-378 has been implicated in myogenesis in C2C12 cells by repressing MyoR, a negative upstream regulator of the transcription factor MyoD (Gagan et al., 2011). Low responders to RE-induced lean mass gain after 12 weeks of RE training show reduced basal miR-378 expression, whereas miR-378 expression is unchanged in those classified as high responders (Davidsen et al., 2011). The change in miR-378 expression has also been correlated ($r^2 = 0.52$) with lean mass

gain after RE training (Davidsen et al., 2011), which suggests maintenance of miR-378 expression may be necessary for promoting RE-induced muscle growth. Concurrent exercise incorporating HIT, but not MICT, reduced miR-378 expression at +1 h relative to that induced by RE alone. However, whether this early post-exercise reduction in miR-378 expression displays a similar relationship with lean mass gain following short-term training, as do changes in basal miR-378 expression (Davidsen et al., 2011), remains to be determined.

miR-486 has been linked to the regulation of skeletal muscle mass by targeting components of the Akt pathway, including PTEN (phosphatase and tensin homologue), an upstream inhibitor of Akt, and by negatively regulating the transcription factor Fox-O1 (Xu et al., 2012), a mediator of ubiquitin ligase expression and subsequently protein degradation (Sandri et al., 2004). Increased post-exercise miR-486 expression, when repeated over time, may therefore be favourable for promoting anabolism in skeletal muscle by alleviating PTEN-mediated repression of Akt, and suppressing Fox-O1-mediated ubiquitin ligase expression. In the present study, miR-486 expression was decreased at +1 h for the HIT+RE trial compared with RE alone, potentially suggesting reduced miR-486-mediated inhibition of PTEN and Fox-O1. Whether this early post-exercise response translates into downstream effects on PTEN and Fox-O1 protein levels is, however, difficult to determine within the time course of the present study. We observed increased, rather than decreased, Fox-O1 mRNA expression for the HIT+RT trial compared with MICT+RT. This still does not discount the possibility that miR-486 may mediate its effects by either degrading Fox-O1 mRNA at a later timepoint, or by repressing its translation. Further work using extended time courses is required to investigate whether these early post-exercise changes in miRNA expression with concurrent exercise are associated with changes in predicted target mRNA and/or protein levels. Taken together, these data suggest concurrent exercise incorporating HIT, but not MICT, alters the post-exercise expression of miRNAs purported to play a role in exercise-induced adaptations in skeletal muscle. However, further work is required to determine the functional roles of these miRNAs in human skeletal muscle with exercise, and their subsequent effects on skeletal muscle adaptations after prolonged training.

3.5.1 Limitations

As reported in similar studies investigating molecular interference with concurrent training (Apro et al., 2013; Coffey et al., 2009a; Coffey et al., 2009b; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015), the phosphorylation of components of the mTORC1 pathway was used as a proxy for the activation of protein synthesis. However, a direct coupling between mTORC1 signalling and protein synthesis rates does not always exist (Atherton et al., 2010), nor does greater phosphorylation of mTOR pathway intermediates always equal a greater protein synthesis response (Crozier et al., 2005). Even functional measures, such as rates of MPS after a single bout of RE, do not correlate with muscle hypertrophy after 16 weeks of RE (Mitchell et al., 2014). Further work is required to define the molecular events that mediate chronic phenotypic adaptations to training, and potentially their progression during a training program. Such information is likely to also provide further mechanistic insight into the concurrent interference effect (Fyfe et al., 2014).

All muscle analyses reported herein were conducted using mixed whole-muscle homogenate, which may have masked any fibre-type specific differences in muscle glycogen content, signalling responses and gene expression (Murphy & Lamb, 2013). Indeed, whilst the HIT and MICT protocols induced similar whole-muscle glycogen depletion and markers of AMPK activity, there might be fibre-type specific differences in these responses (Suriano et al., 2010). Thus, whether the prior endurance exercise protocols altered signalling responses and/or gene expression in a fibre-type specific manner following subsequent RE remains to be determined.

A pre-exercise feeding protocol was employed in an attempt to mimic typical exercise/nutritional scenarios by not performing exercise in a fasted state, an approach used previously in similar studies (Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015). This feeding protocol was purposely low in protein content (~0.1 $g \cdot kg^{-1}$), thereby limiting the potential impact of amino acid provision on inducing anabolic signalling responses. While this feeding approach may better mimic typical training scenarios, it may limit the ability to compare the present results to previous studies examining molecular responses in skeletal muscle to concurrent exercise performed in the fasted state (Apro et al., 2015; Apro et al., 2013; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012).

3.5.2 Conclusions

This is the first investigation to directly compare the effects of HIT and work-matched MICT on mTORC1 signalling and miRNA expression in human skeletal muscle when performed concurrently with RE. It was shown that although HIT and MICT similarly reduce muscle glycogen content and increased markers of AMPK activity prior to a subsequent RE bout, post-exercise mTORC1 signalling is generally not compromised compared with RE performed alone. However, concurrent exercise incorporating HIT or MICT similarly increased MuRF-1 expression relative to single-mode RE, potentially indicating increased rates of protein degradation. Concurrent exercise incorporating HIT, but not MICT, also reduced the post-exercise expression of miRNAs linked to the regulation of skeletal muscle hypertrophy. Taken together, the present results suggest that HIT is a potent stimulus for inducing anabolic responses in skeletal muscle after concurrent exercise, including increased mTOR and rps6 phosphorylation, and for altering the expression of miRNAs implicated in adaptations to RE, at least in relatively training-unaccustomed subjects. Further work is required to link the modulation of these early post-exercise anabolic responses to phenotypic training adaptations, as well as the efficacy of incorporating either HIT or MICT into long-term concurrent training programs.

Chapter 4. Physiological and morphological adaptations to eight weeks of concurrent training incorporating high-intensity interval or work-matched continuous endurance training

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4.1 Linking statement

Chapter 3 (Study 1) investigated the early molecular events in skeletal muscle after single bouts of concurrent exercise, incorporating either HIT or MICT as the endurance exercise modality, compared with RE undertaken alone. It is clear from these data, which supports other recent evidence, that early post-exercise translation initiation signalling (i.e., mTORC1 signalling) is not compromised when RE is combined with endurance exercise. These data also highlight the ability of endurance exercise to promote anabolic signalling responses in skeletal muscle, and corroborate earlier evidence showing little divergences in AMPK or mTORC1 signalling to single bouts of either endurance or RE performed separately. Nevertheless, there is limited evidence for any substantial relationship between the phosphorylation of signalling proteins following early exercise bouts, or even direct measures of MPS, and long-term training adaptations such as muscle hypertrophy or maximal strength gain. This suggests that examining early molecular responses to single bouts of exercise, particularly in relatively untrained individuals, may provide limited insight into long-term adaptation and indeed interference with concurrent training. Longer-term training studies are therefore required to more definitively evaluate the effects of altering concurrent training variables on long-term training adaptations. These data will help to elucidate whether there is a role for different training variables, including endurance training intensity, in mediating any interference effect during concurrent training. The aim of the following chapter was therefore to elucidate whether concurrent training incorporating HIT or work-matched MICT modulates interference to RT adaptations when compared with an identical RT paradigm performed alone.

4.2 Introduction

Simultaneously incorporating both endurance and resistance training (RT) into a periodised exercise program is termed concurrent training. Compared with RT alone, concurrent training has been reported to attenuate training-induced improvements in maximal strength, power, and skeletal muscle hypertrophy in most (Bell et al., 2000; Craig et al., 1991; Hennessy & Watson, 1994; Hickson, 1980; Kraemer et al., 1995), but not all (Balabinis et al., 2003; McCarthy et al., 2002), studies. The equivocal nature of this interference effect can possibly be attributed to between-study variations in the prescription of individual training variables, which may modulate the degree of interference seen with concurrent training (Fyfe et al., 2014).

Two training variables likely to be important in mediating the interference effect are endurance training intensity and/or volume (Fyfe et al., 2014; Wilson et al., 2012). Endurance training intensity is a particularly relevant practical consideration, given that high-intensity interval training (HIT) can be more effective for enhancing aerobic capacity (Milanovic et al., 2015), and reducing cardiometabolic risk factors (Tjonna et al., 2008; Wisloff et al., 2007), compared with traditional moderate-intensity continuous training (MICT). Evidence also suggests that HIT protocols involving brief work intervals (~2-4 min) interspersed with periods of active or passive recovery (~1-3 min) are perceived as more enjoyable compared with MICT (Bartlett et al., 2011), and are well-tolerated in clinical populations (Tjonna et al., 2008; Wisloff et al., 2007). Thus, HIT represents an attractive exercise strategy for improving aerobic capacity and cardiometabolic risk factors compared with MICT across a variety of populations, and with promising implications for exercise adherence.

Despite the efficacy of HIT for promoting positive health and performance outcomes (Milanovic et al., 2015; Tjonna et al., 2008; Wisloff et al., 2007), there is currently limited information on the effects of incorporating HIT compared with MICT into concurrent training programs. Indeed, studies independently examining the potential role of endurance training intensity upon interference during concurrent training are scarce (Silva et al., 2012). One study (Silva et al., 2012) simultaneously investigated the effects of endurance training intensity (i.e., continuous vs. interval training) and modality (i.e., cycling vs. running) on neuromuscular adaptations to eleven weeks of concurrent training in physically-active females. No differences for

improvements in one-repetition maximum (1-RM) leg press strength were found between training groups performing either RT only (52.6%) or concurrent training incorporating either continuous cycling (39.1%), continuous running (41.1%), or interval running (46.8%). However, the endurance training protocols used were only matched for total exercise duration, and not total work, making it difficult to deduce any potential influence of training intensity in mediating any effect on training-induced maximal strength outcomes (Silva et al., 2012). Further work is therefore required to delineate the potential roles of endurance training intensity on interference to maximal strength, power and hypertrophy outcomes during concurrent training.

Concurrent endurance training may interfere with RT adaptations by either i) compromising subsequent RT performance via exacerbating residual fatigue and/or substrate depletion, or ii) attenuating post-exercise anabolic responses that govern increases in rates of muscle protein synthesis and subsequent muscle fibre hypertrophy (Fyfe et al., 2014). A single bout of high-intensity endurance exercise reduces force generating capacity of the exercised musculature for at least six hours post-exercise (Bentley et al., 2000), with lower-intensity training reported to elicit less residual fatigue (de Souza et al., 2007; Leveritt et al., 2000). Prior endurance exercise also compromises subsequent RT performance by reducing maximal strength or limiting RT volume (de Souza et al., 2007; Tan et al., 2014), an effect exacerbated after higher-intensity interval compared with lower-intensity continuous endurance exercise (de Souza et al., 2007). Higher exercise intensities are also associated with further increases in the activity of kinases purported to limit muscle protein synthesis, including AMPK (5’ adenosine monophosphate-activated protein kinase) (Rose et al., 2009b). Whether these factors render HIT a suitable endurance training strategy to employ during concurrent training, compared with MICT, with respect to modulating interference to RT adaptations is therefore unclear.

Given the efficacy of HIT compared with traditional MICT for improving aerobic capacity and metabolic health markers, the aim of this study was to determine the effect of eight weeks of concurrent training incorporating either HIT or MICT on maximal strength, counter-movement jump (CMJ) performance, and body composition adaptations, compared with single-mode RT, in recreationally-active males. It was hypothesised that, compared with RT performed alone, i) concurrent training incorporating either HIT or MICT would attenuate increases in maximal strength, CMJ performance and lean mass, and ii), this interference effect would be exacerbated when

RT was combined with HIT, compared to with MICT. Identification of training variables that are critical mediators of the interference effect will allow for targeted exercise prescription to minimise interference during concurrent training.

4.3 Methodology

4.3.1 Participants

Twenty-three recreationally-active males (mean ± SD: age, 29.6 ± 5.5 y; height, 182.4 ± 5.9 cm; body mass, 84.9 ± 11.4 kg) completed this investigation (see table 1 for baseline characteristics for each training group). Power calculations using G*Power 3.1 software indicated a total sample size of 24 participants (8 participants per group) was required to detect a small effect (d = 0.35) (Rhea, 2004) for pre-to-post changes and between-group differences in strength variables in recreationally-active individuals, according to the scale suggested by Rhea (2004). A flow chart of the progression of participants through initial participant screening, group randomisation, and to the final sample size included for each training group is shown in Figure 4.1. Participants were undertaking recreational exercise involving aerobic and/or resistance exercise at least twice per week for >30 min, and were free from any current cardiovascular abnormalities or musculoskeletal injuries to the upper or lower extremity. After being fully informed of study procedures (Appendix B) and screening for possible exclusion criteria (Appendices C and F), participants provided written informed consent (Appendix E). All procedures were approved by the Victoria University Human Research Ethics Committee.

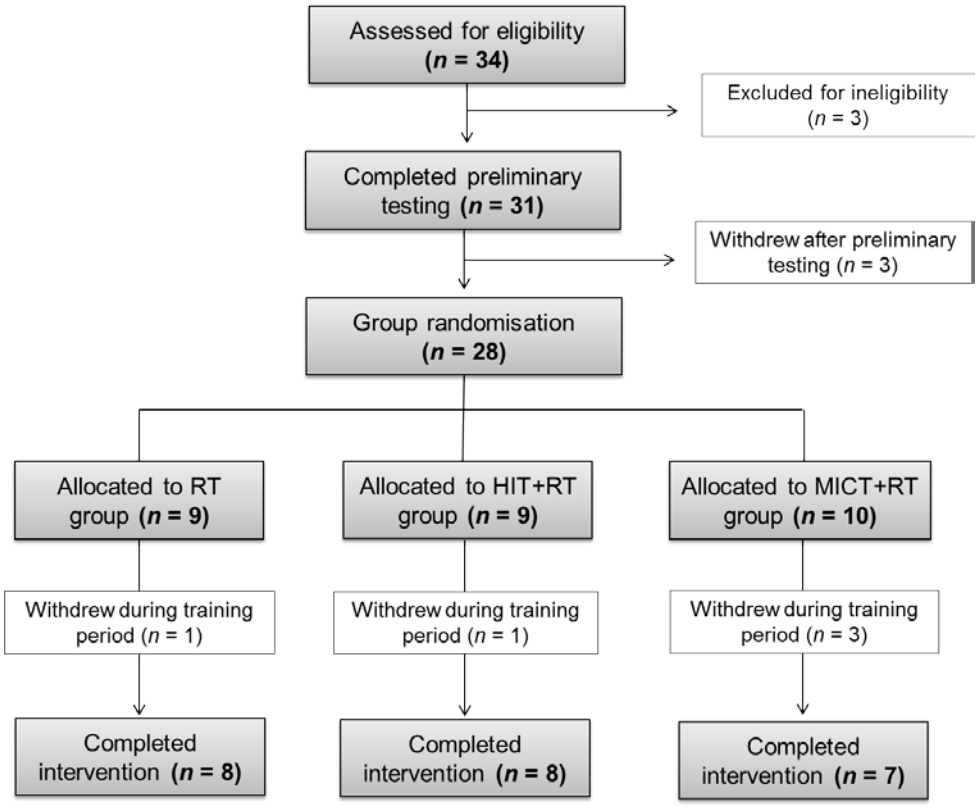

Figure 4.1 Flow chart of progress of participants through initial participant screening, preliminary testing, group randomisation and final sample size for each training group. HIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training.

4.3.2 Study overview

The study followed a repeated-measures, parallel-group design. After preliminary testing, participants were ranked by baseline 1-RM leg press strength and randomly allocated to one of three training groups. Training groups consisted of 1) HIT cycling combined with RT (HIT+RT group, *n* = 8), 2) MICT cycling combined with RT (MICT+RT group, *n* = 7) and 3) RT peformed alone (RT group, *n* = 8). Measures of aerobic capacity, maximal strength, and CMJ performance were obtained before (PRE), mid-way through (MID) and after completion (POST) of the training intervention (Figure 4.2). Body composition analysis (DXA) was performed only at PRE and POST. After preliminary testing, participants completed 8 weeks of group-specific training performed three times per week.

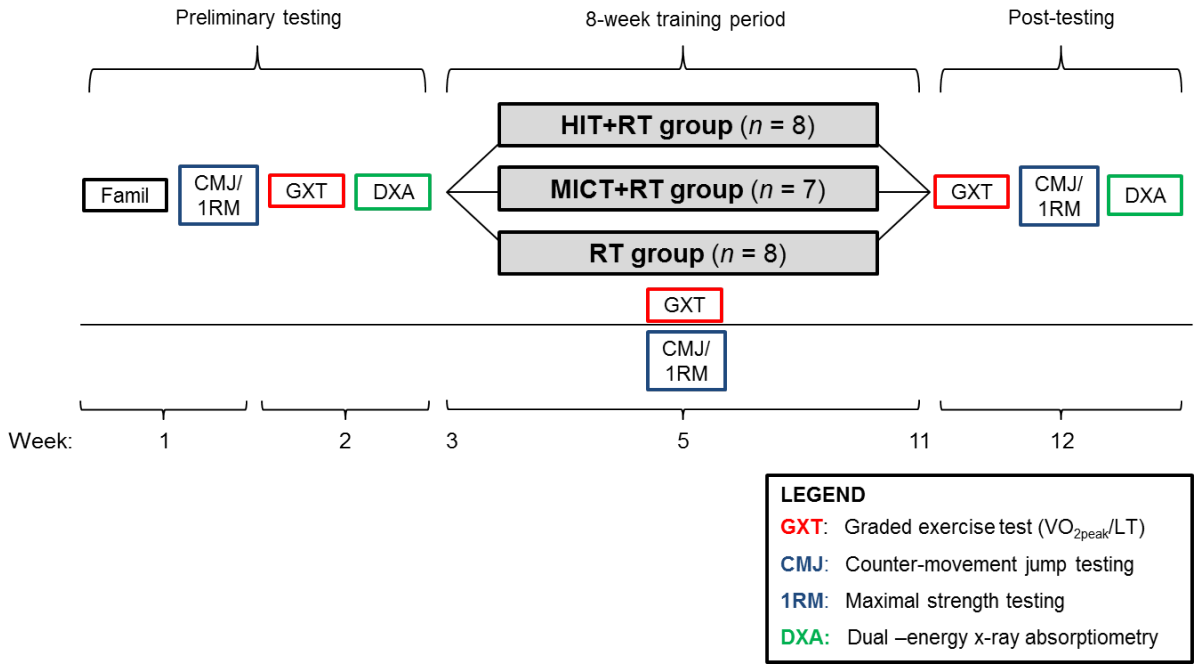

Figure 4.2 Experimental overview for Study 2. HIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training; LT, lactate threshold; GXT, graded exercise test; CMJ, counter-movement jump; 1-RM, one-repetition maximum; Famil, familiarisation; DXA, dual-energy x-ray absorptiometry.

4.3.3 Preliminary testing

4.3.3.1 Familiarisation

Approximately 3-4 days before beginning preliminary testing, participants were familiarised with the CMJ, one-repetition maximum (1-RM) strength and graded exercise test (GXT) protocols (each described subsequently).

4.3.3.2 Diet & exercise control

For 24 h prior to the GXT, CMJ/1-RM testing and DXA, participants refrained from any structured exercise and recorded a detailed food diary (Appendix G). Participants were then asked to replicate this dietary intake as accurately as possible for the 24 h prior to each respective post-training test. On the morning of all testing sessions, participants reported to the laboratory after an ~8-10 h overnight fast. Prior to commencement of the training intervention, participants were asked to record a detailed 72 h food diary (Appendix H) for the purposes of calculating average daily habitual energy and macronutrient intake. Dietary recalls were analysed using Foodworks

software (Version 6.0, Xyris Software, Australia). During the intervention period, participants were asked to maintain habitual dietary practices as closely as possible.

4.3.3.3 Graded exercise test (GXT)

The lactate threshold (LT) and peak aerobic power (W_{peak}) were obtained during a GXT performed to volitional exhaustion on an electromagnetically-braked cycle ergometer (Lode, Groningen, The Netherlands). Prior to the GXT, a venous catheter was inserted into an antecubital forearm vein for subsequent blood sampling. The GXT consisted of 4-min work stages interspersed with 30 s of passive recovery. Participants maintained a pedalling cadence of 70 rpm during each work stage. The initial workload was set at 60, 90 or 120 W (to limit the number of stages to a maximum of 10, as determined during familiarisation), and increased by 30 W for each subsequent stage until volitional exhaustion, defined as an inability to maintain a cadence >60 rpm. Venous blood samples (~1 mL) were drawn from the cannula at rest, and immediately following completion of each work stage. Whole-blood samples were immediately analysed in duplicate for lactate concentration using an automated analyser (YSI 3000 STAT, Yellow Springs, OH). The lactate threshold was defined as the first workload that elicited a >1 mM increase in venous blood lactate concentration from baseline (Coyle et al., 1983) and was calculated using Lactate-OR software (Newell et al., 2007). The W_{peak} was determined as previously described (Hawley & Noakes, 1992).

4.3.3.4 Peak oxygen uptake ($\dot{V}O_{2peak}$) test

Immediately following the GXT, a 5-min active recovery was initiated at 20 W, after which participants again cycled to volitional exhaustion at a workload corresponding to 105% of the W_{peak} achieved during the GXT. Participants were instructed to accelerate to a cadence of 90-100 rpm upon a 5-s countdown, and the test terminated when a cadence >60 rpm was no longer possible. Expired gases were sampled every 15 s during this test component using automated gas analysers (Moxus Modular $\dot{V}O_2$ System, AEI Technologies, Pittsburgh, PA). A similar protocol has previously been reported to elicit $\dot{V}O_{2peak}$ values no different to that determined during a ramp incremental test performed 5 min previously (Rossiter et al., 2006). The gas analysers and pneumotach were calibrated prior to each test using known gas concentrations (21.0% O_2 and 0.04% CO_2, 16.0% O_2 and 4.0% CO_2) and a 3-L

calibration syringe, respectively. The individual $\dot{V}O_{2peak}$ was defined as the highest two consecutive 15-s values achieved during the test. The test-retest reliability of GXT variables has been previously determined during repeated testing in our laboratory and yielded the following typical error values (expressed as a coefficient of variation [CV] ±90% confidence intervals): LT (6.8 ±1.2%), W_{peak} (5.5 ±1.2%), and $\dot{V}O_{2peak}$ (6.5 ±1.2%).

4.3.3.5 Maximal strength (1-RM) testing

Maximal strength was determined during a series of one-repetition maximum (1-RM) leg press and bench press attempts using a plate-loaded 45° incline leg press (Hammer Strength Linear, Schiller Park, IL) and standard bench press, respectively. After a standardised warm-up (5 and 3 repetitions at 50 and 70% estimated 1-RM, respectively), single repetitions of increasing load were attempted until the maximal load possible for one repetition was determined. Three minutes of recovery was allowed between 1-RM attempts. For the leg press, each repetition began in full knee extension with the heel placed at the bottom edge of the foot plate, and with a range of motion of 90° knee flexion/extension. Bench press repetitions were initiated from a position of full elbow extension, after which the barbell was lowered to the position of the chest and again lifted to full elbow extension. The test-retest reliability of 1-RM testing using similar protocols as the present study has been reported previously, with typical error values (expressed as a coefficient of variation [CV]) of 3.3% (Levinger et al., 2009) and 2.8% (McGuigan & Winchester, 2008) for 1-RM leg press and bench press, respectively.

4.3.3.6 Counter-movement jump (CMJ) testing

Counter-movement jump (CMJ) performance was assessed using a force plate (Fitness Technology, Skye, SA) interfaced with a linear position transducer (Ballistic Measurement System, Fitness Technology, Skye SA). After a standardised warm-up protocol (three submaximal unloaded CMJs), participants performed three maximal unloaded CMJs on the force plate with one min of passive recovery between each effort. The best of three trials were chosen for analysis. Jumps were initiated from a standing starting position, with the hands placed on the hips throughout the jump. Participants were instructed to self-select their jump depth and then accelerate as quickly as possible

from the bottom position to achieve maximal concentric velocity and jump height. To allow for direct measurement of vertical displacement and movement velocity during each jump, the linear position transducer was attached to the centre of mass of each participant via a weight belt. The test-retest reliability of CMJ variables was determined between the familiarisation and preliminary testing sessions and yielded the following typical error values (expressed as a CV ±90% confidence intervals): peak CMJ force (5.4 ±1.5%), peak CMJ power (4.3 ±1.5%), peak CMJ displacement (5.9 ±1.5%), and peak CMJ velocity (3.7 ±1.5%).

4.3.3.7 Body composition

Body composition was assessed via dual-energy x-ray absorptiometry (DXA; Discovery W, Hologic Inc., Bedford, MA) both pre- and post- training. DXA is a valid and reliable measurement tool for estimating total and regional body fat and lean mass (Nana et al., 2012). Typical error of measurement for regional lean mass measured via DXA has been reported as 1.3-1.7% (Nana et al., 2012) with strict control of diet and body position, while typical error for total lean and fat mass has been reported as 0.5% and 1.3%, respectively (Nana et al., 2012). To improve measurement reliability, participants were scanned in the fasted state and asked to refrain from exercise for 24 h before each scan. The scanner was calibrated daily, and the same certified densitometry technician performed and analysed both the PRE and POST scans for each participant.

4.3.4 Training intervention

Participants began the 8-week training intervention 3-5 days after completion of preliminary testing. All training groups performed an identical RT program on non-consecutive days (typically Monday, Wednesday, and Friday), with the HIT+RT and MICT+RT groups completing the corresponding form of endurance exercise 10 min prior to commencing each RT session. The order in which concurrent endurance and resistance exercise sessions were performed, as well as the between-mode recovery period employed, were chosen to create a likely sub-optimal scenario for the RT-induced development of muscle mass and strength (potentially due to exacerbated residual fatigue and/or increased skeletal muscle AMPK activity induced by prior endurance exercise). All training programs were progressively modified to provide a sufficient overload stimulus, and are described in detail subsequently.

4.3.4.1 Endurance training

All cycling training sessions began with a 5-min warm-up performed at 75 W. The HIT protocol involved multiple 2-min intervals performed on an electromagnetically-braked cycle ergometer (Velotron RacerMate, Seattle, WA) at an intensity ranging between 120-150% of the LT, interspersed with 1 min of passive recovery. The MICT protocol involved continuous cycling performed on an electromagnetically-braked cycle ergometer (Velotron RacerMate, Seattle, WA) for a duration of between 15 and 33 min, and at a relative intensity ranging between 80-100% of the LT. All MICT sessions were work- and duration-matched to the corresponding HIT session (Edge et al., 2006). Progressive overload was applied by modulating the the number of intervals and relative exercise intensity (HIT) and the duration of cycling and relative exercise intensity (MICT) throughout the training program (Table 4.1). After re-testing of the GXT protocol at MID, relative endurance training intensities were adjusted as a percentage of the MID-training LT.

Table 4.1 Progression of HIT and MICT prescription throughout the eight-week training intervention for Study 2. HIT, high-intensity interval training; MICT, moderate-intensity continous training; LT, lactate threshold.

		HIT		MICT	
Week	Session	No. of 2-min intervals	Training intensity (% LT)	Duration of continuous training (min)	Training intensity (% LT)
1	1	5	120	15	80
	2	6	120	18	80
	3	7	120	21	80
2	1	6	120	18	80
	2	8	120	24	80
	3	7	120	21	80
3	1	8	130	24	86.7
	2	9	130	27	86.7
	3	8	130	24	86.7
4	1	7	130	21	86.7
	2	6	130	18	86.7
	3	5	130	15	86.7
5	1	7	140	21	93.3
	2	8	140	24	93.3
	3	9	140	27	93.3
6	1	8	140	24	93.3
	2	9	140	27	93.3
	3	10	140	30	93.3
7	1	9	150	27	100
	2	11	150	33	100
	3	10	150	30	100
8	1	9	150	27	100
	2	7	150	21	100

4.3.4.2 Resistance training (RT)

The RT program was performed three times per week on non-consecutive days. Sessions 1 and 3 of each training week included the leg press, bench press, seated row, leg extension and leg curl exercises. Session 2 of each training week included the leg press, flat dumbbell press, lat pulldown, dumbbell lunges and leg curl exercises. All exercises were performed at an intensity of between ~65-90% 1-RM (14- to 4-RM), with 2-3 min of recovery allowed between sets. For exercises where the 1-RM was not determined, load prescription was based on the maximum number of repetitions possible for a given load (i.e., the n-RM). For example, training prescription was set at 12 repetitions with a 14-RM load during the first week of training. During the first training session, loads were therefore adjusted until no more than 14 repetitions were possible with a given load for each exercise. During subsequent sessions, training loads were then increased concomitantly with changes in the n-RM prescription (Table 4.2). For each exercise, participants were instructed to perform the concentric portion of each repetition with a near-maximal to maximal intended movement velocity. The first three exercises of each session were preceded by a single warm-up set performed at approximately 75% of the planned workload for each respective exercise. Progressive overload was applied by altering the number of sets, repetitions, duration of rest periods, and relative exercise intensities throughout the training program (Table 4.2).

Table 4.2 Progression of resistance training prescription throughout the eight-week training intervention for Study 2. RM, repetition maximum; 1-RM, one-repetition maximum.

Mon/Fri program	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Sets × repetitions	3 × 12	3 × 10	3 × 8	3 × 6	4 × 6	4 × 6	4 × 4	5 × 4
RM load	14	12	9	7	7	7	4	4
Rest period (min)	2	2	2	3	3	3	3	3
% 1-RM load	65	70	77.5	82.5	82.5	87.5	90	90
Wed program								
Sets × repetitions	3 × 12	3 × 12	3 × 10	3 × 10	3 × 8	3 × 8	4 × 6	3 × 6
RM load	14	14	12	12	9	9	7	7
Rest period (min)	2	2	2	2	2	2	2	2
% 1-RM load	65	65	70	70	77.5	77.5	87.5	87.5

4.3.5 Physiological and psychological responses to exercise

To quantify the physiological and psycholigical responses to HIT and MICT, exercise heart rate (HR; Polar Electro, Kempele, Finland) and rating of perceived exertion (RPE; Borg 6-20 scale) responses were collected at regular intervals during HIT and MICT sessions conducted in the first session of training weeks 1, 4, 5 and 8. For HIT sessions, HR and RPE data were collected after completion of each 2-min interval, while for MICT these data were collected at the equivalent time points during continuous exercise.

4.3.6 Training load quantification

Internal (i.e., perceived) training loads were quantified during the intervention period using the session RPE (sRPE) method (Foster et al., 2001). The sRPE method is a valid and reliable tool for quantifying internal training load for both endurance (Foster et al., 2001) and resistance exercise (Day et al., 2004). For the HIT+RT and MICT+RT groups, the sRPE for cycling was obtained 10 min following each cycling session (i.e., immediately prior to subsequent resistance exercise), to determine training load for the HIT and MICT protocols. For all training groups, the sRPE was also obtained within 10 min after completion of RT as a marker of total session training load. In addition to quantifying the prescribed training load, we also monitored training load for all exercise completed by participants outside of the study during the 8-week training intervention period (i.e., non-prescribed training load) using a custom, web-based training diary. Participants were asked to record the sRPE, duration, and description of the activity within 30 min of completing each non-prescribed external training session. The non-prescribed training load was then added to the prescribed training load to determine the combined internal training load experienced by participants during the training intervention.

4.3.7 Statistical analyses

The effect of training group on outcomes was evaluated via a two-way (time × group) analysis of variance with repeated-measures (RM-ANOVA) (SPSS, Version 21, IBM Corporation, New York, NY). Outcome variables were log-transformed before analysis to reduce non-uniformity of error (Hopkins et al., 2009). A magnitude-based

approach to inferences using the standardised difference (effect size, ES) was used as previously described (Hopkins et al., 2009), with the default threshold of 0.2 defined as the smallest worthwhile change. Magnitude-based inferences about effects were made by qualifying the effects with probabilities that reflected the uncertainty in the magnitude of the true effect (Batterham & Hopkins, 2005); 25-75%, possibly; 75-95%, likely; 95-99.5%, very likely; >99.5%, most likely. Effects deemed at least 75% 'likely' to be substantial (according to the overlap between the effect magnitude, the uncertainty in the magnitude of the true effect, and the smallest worthwhile change (Batterham & Hopkins, 2005)) were included for analysis. Exact *P* values were also determined for each comparison, derived from paired (for within-group comparisons) or unpaired (for between-group comparisons) *t*-tests, with a Bonferroni correction applied to correct for multiple comparisons (SPSS, Version 21, IBM Corporation, New York, NY). All data are reported as the mean change (from PRE) ±90% CL, unless otherwise specified.

4.4 Results[3] [4]

4.4.1 Training compliance

There were no between-group differences in training compliance (% of total sessions completed; mean ± SD: HIT+RT, 98 ± 3%; MICT+RT, 97 ± 4%; RT, 98 ± 2%).

4.4.2 Physiological and psychological responses to HIT and MICT

Average HR was higher during HIT compared with MICT during the first training session conducted in weeks 1, 4, and 5 (mean difference range ±90% confidence interval, 13 ±8 to 16 ±10 beats·min^{-1}; ES range ±90% confidence interval, 1.29 ±0.85 to 1.45 ±0.90; $P \leq 0.024$). Similarly, average RPE was also higher for HIT compared with MICT during the first training session conducted in weeks 1, 4, 5 and 8 (2 ±1 to 3 ±2 AU; ES, 0.98 ±0.86 to 1.49 ±0.90; $P \leq 0.067$).

[3] All raw data for this Chapter is available in Appendix L.
[4] Extended within- and between-group comparison data for this chapter are presented in Appendices O and P, respectively.

4.4.3 Training load

4.4.3.1 Weekly training load (cycling only)

There were main effects of time ($P < 0.001$), group ($P = 0.005$), and a time × group interaction ($P = 0.003$) for cycling-only weekly training load. Cycling-only weekly training load (Figure 4.3A) was higher for HIT compared with MICT during training weeks 1-7 (% weekly difference range ±90% confidence interval, 23 ±15 to 49 ±24%; ES range ±90% confidence interval, 1.21 ±0.87 to 2.07 ±0.90; $P \leq 0.023$).

4.4.3.2 Weekly training load (total session)

There were main effects of time ($P < 0.001$), group ($P < 0.001$), and a time × group interaction ($P < 0.001$) for total session weekly training load. Total session weekly training load (Figure 4.3B) was higher during all training weeks for both HIT+RT (72 ±30 to 244 ±85%; ES, 2.77 ±0.84 to 5.55 ±0.89; $P < 0.001$) and MICT+RT (19 ±34 to 302 ±61%, ES, 0.32 ±0.92 to 8.20 ±0.89; $P < 0.002$) compared with RT. Total session weekly training load was also higher for HIT+RT compared with MICT+RT at week 1 (31 ±35%; ES, 0.70 ±0.90; $P < 0.001$), week 2 (15 ±22%; ES, 0.58 ±0.90; $P < 0.001$), week 3 (13 ±20%; ES, 0.54 ±0.89; $P = 0.007$), and week 7 (19 ±14%; ES, 1.09 ±0.89; $P = 0.004$).

4.4.3.3 Total study training loads

There were main effects of group for differences in total study prescribed training load ($P < 0.001$) and total study combined (i.e., prescribed + non-prescribed) training load ($P = 0.001$). Total study prescribed training load (Figure 4.3C) was higher for both HIT+RT (119 ±44%; ES, 3.26 ±0.84; $P < 0.001$) and MICT+RT (108 ±40%; ES, 3.28 ±0.87; $P < 0.001$) compared with RT. Total study non-prescribed training load (Figure 4.3C) was higher HIT+RT compared with both RT (278 ±624%; ES, 0.94 ±0.92; $P = 0.077$) and MICT+RT (66.8 ±49.9%; ES, 0.81 ±0.87; $P = 0.116$). Total study combined (i.e., prescribed + non-prescribed) training load (Figure 4.3C) was higher for both HIT+RT (173 ±72%; ES, 3.21 ±0.84; $P < 0.001$) and MICT+RT (108 ±70%; ES, 1.94 ±0.87; $P = 0.001$) compared with RT. Total study combined training load was also higher for HIT+RT compared with MICT+RT (24 ±25%; ES, 0.73 ±0.88; $P = 0.150$).

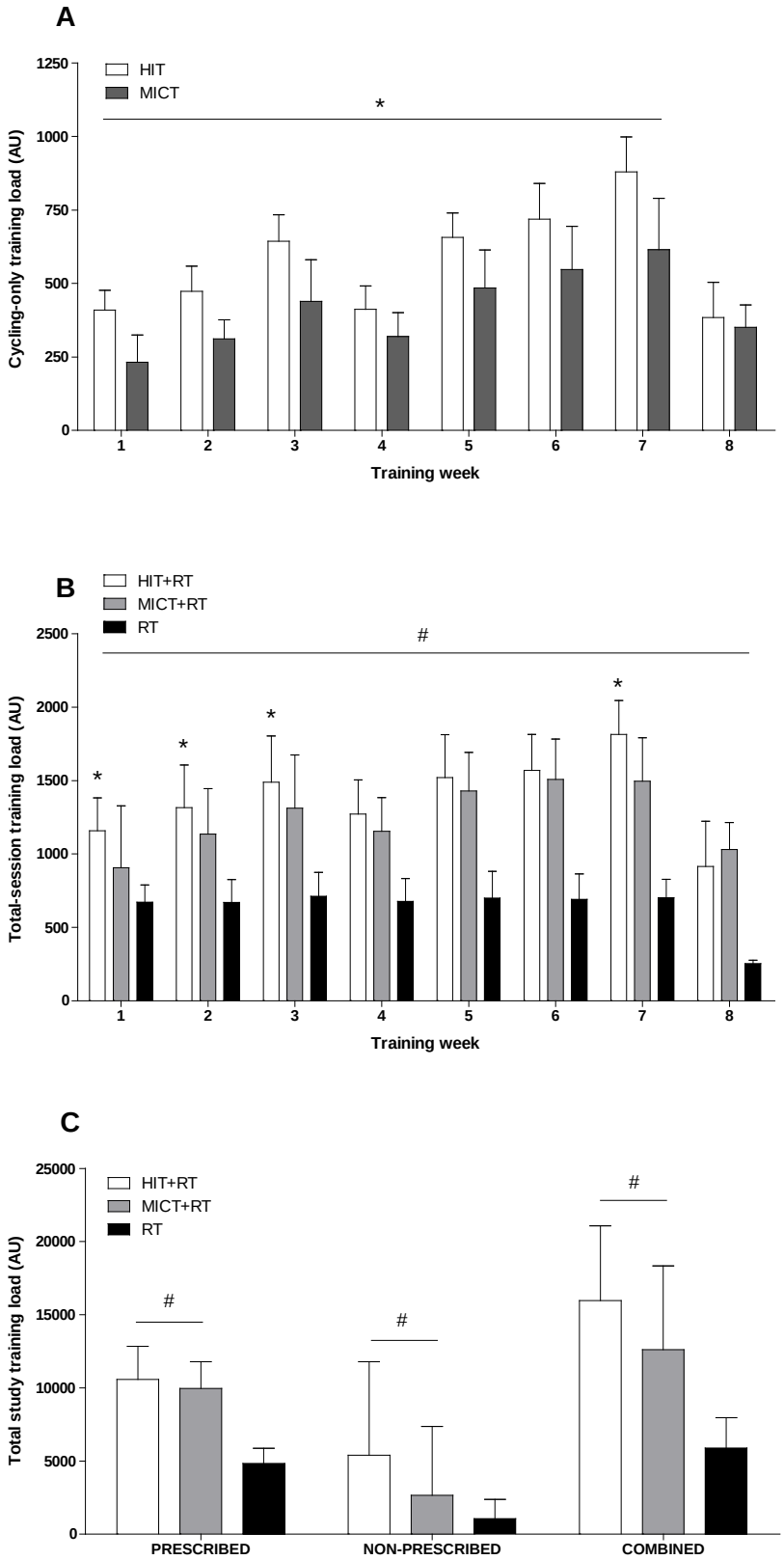

Figure 4.3 Weekly cycling only (A) and total session (B) internal training load, and total prescribed, non-prescribed, and combined (prescribed + non-prescribed) internal training loads (C) during the 8-week training period for all training groups. HIT, high-intensity interval training; MICT, moderate-intensity continous training; RT, resistance training. * = *P* < 0.05 vs. MICT; # = *P* < 0.05 vs. RT. Data shown are means ± SD.

4.4.4 Habitual dietary intake

There was a main effect of group for differences in baseline average daily fat intake (P = 0.035). There were no substantial between-group differences in average daily protein intake at baseline (RT: 1.11 ± 0.37 g·kg^{-1}·day^{-1}; HIT+RT: 1.29 ± 0.34 g·kg^{-1}·day^{-1}; MICT+RT: 1.14 ± 0.28 g·kg^{-1}·day^{-1}; P > 0.05). Average total energy intake was, however, higher for HIT+RT compared with RT (1079 ±1369 kJ·day^{-1}; ES, 0.66 ±0.84; P = 0.208) due largely to a higher fat intake for HIT+RT compared with both RT (23.4 ±20.9 g·day^{-1}; ES, 0.77 ±0.84; P = 0.907) and MICT+RT (27.8 ±19.9 g·day^{-1}; ES, 1.02 ±0.85; P = 0.246). Average daily carbohydrate intake was also higher for MICT+RT compared with both HIT+RT (26.3 ±37.8 g·day^{-1}; ES, 0.57 ±0.89; P = 0.058) and RT (27.1 ±38.9 g·day^{-1}; ES, 0.58 ±0.88; P = 0.744).

4.4.5 Maximal strength

4.4.5.1 1-RM leg press strength

There was a main effect of time for changes in 1-RM leg press strength (P < 0.001; Figure 4.4A), which was improved from PRE to POST for RT (mean difference ±90% CL, 38.5 ±8.5%; ES ±90% CL, 1.26 ±0.24; P < 0.001), HIT+RT (28.7 ±5.3%; ES, 1.17 ±0.19; P < 0.001) and MICT+RT (27.5 ±4.6%, ES, 0.81 ±0.12; P < 0.001). The change in 1-RM leg press strength from PRE to POST was greater for RT compared with HIT+RT (7.4 ±8.7%; ES, 0.40 ±0.40) and MICT+RT (8.2 ±9.9%; ES, 0.60 ±0.45), with trivial differences in this response between HIT+RT and MICT+RT (0.9 ±8.1%; ES, 0.03 ±0.30).

4.4.5.2 1-RM bench press strength

There was a main effect of time for changes in 1-RM bench press strength (P < 0.001; Figure 4.4B), which was improved from PRE to POST for RT (20.5 ±6.2%; ES; 0.50 ±0.14; P < 0.001), HIT+RT (15.9 ±2.6%; ES, 0.62 ±0.09; P < 0.001) and MICT+RT (14.8 ±2.3%; ES, 0.39 ±0.06; P < 0.001). There were no substantial differences in the training-induced change in 1-RM bench press between RT and either HIT+RT (1.0 ±4.7%; ES, 0.04 ±0.22) or MICT+RT (4.7 ±6.1%; ES, 0.15 ±0.20).

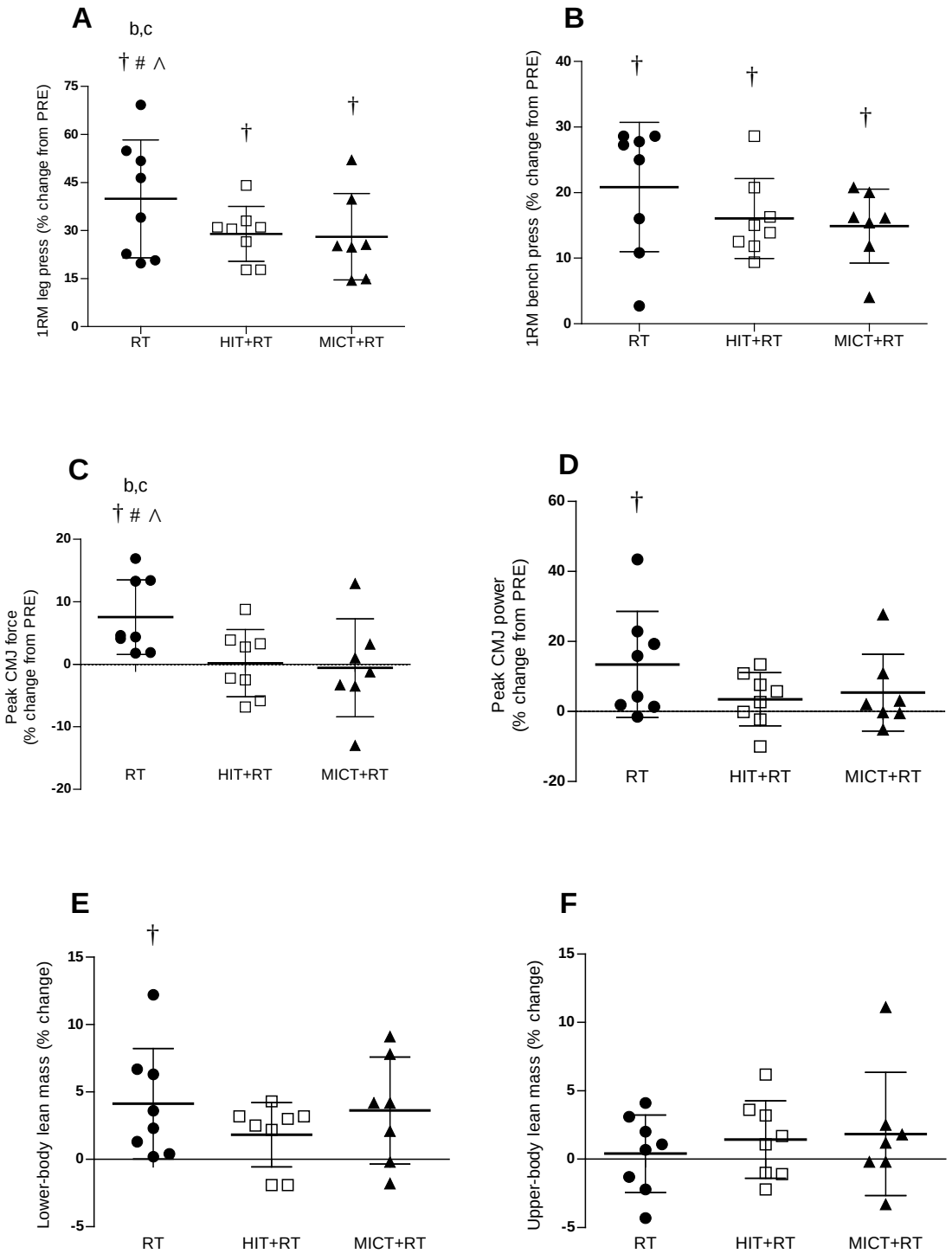

Figure 4.4 Percentage changes in 1-RM leg press (A) and bench press (B) strength, peak counter-movement jump (CMJ) force (C) and power (D), and lower-body (E) and upper-body (F) lean mass between PRE- and POST-training. RT, resistance training; HIT, high-intensity interval training; MICT, moderate-intensity continuous training; 1-RM, one-repetition maximum. Data shown are mean changes ± SD and individual participant responses. † = *P* < 0.05 vs. PRE-training; # = *P* < 0.05 vs. HIT+RT, ^ = *P* < 0.05 vs. MICT+RT. Change from PRE to POST substantially greater vs. b = HIT+RT, c = MICT+RT.

4.4.6 Counter-movement jump (CMJ) performance

There was a time × group interaction (P = 0.041) for changes in peak counter-movement jump (CMJ) force, and main effects of time for changes in peak CMJ power (P = 0.008), peak CMJ velocity (P = 0.012) and peak CMJ displacement (P = 0.007).

4.4.6.1 Peak CMJ force

Peak CMJ force (Figure 4.4C) increased from PRE to POST for RT (7.4 ±3.4%; ES, 0.46 ±0.20; P = 0.008), with this change almost completely attenuated for HIT+RT (0.1 ±3.6%; ES, 0.00 ±0.23; P = 0.979) and MICT+RT (-0.8 ±4.9%; ES, -0.04 ±0.26; P = 0.790). The PRE to POST change in peak CMJ force was also greater for RT compared with HIT+RT (6.8 ±4.5%; ES, 0.41 ±0.28) and MICT+RT (9.9 ±11.2%; ES, 0.54 ±0.65).

4.4.6.2 Peak CMJ power

Peak CMJ power (Figure 4.4D) increased from PRE to POST for RT (12.6 ±10.5%; ES, 1.09 ±0.85; P = 0.035), but not for either HIT+RT (3.2 ±5.6%; ES, 0.20 ±0.34; P = 0.266) or MICT+RT (5.0 ±6.1%; ES, 0.19 ±0.23; P = 0.241). The PRE to POST change in peak CMJ power was, however, not substantially different for RT compared with either HIT+RT (5.1 ±7.3%; ES, 0.38 ±0.56) or MICT+RT (3.5 ±8.7%; ES, 0.21 ±0.54).

4.4.6.3 Peak CMJ velocity

Peak CMJ velocity (Table 4.3) was increased from PRE to POST for RT (9.6 ±8.2%; ES, 0.29 ±0.24; P = 0.099) and MICT+RT (6.0 ±4.0%; ES, 0.40 ±0.26; P = 0.015), but not for HIT+RT (2.6 ±4.8%; ES, 0.17 ±0.31; P = 0.306). There were no substantial between-group differences in the PRE to POST change in peak CMJ velocity.

4.4.6.4 Peak CMJ displacement

Peak CMJ displacement (Table 4.3) was not substantially different between PRE and POST for either RT (9.5 ±10.0%; ES, 0.22 ±0.22; P = 0.108) or MICT+RT

(7.0 ±8.5%; ES, 0.34 ±0.40 *P* = 0.129); however, a likely small increase was noted for HIT+RT (7.8 ±9.1%; ES, 0.50 ±0.56; *P* = 0.134). There were no substantial between-group differences in the PRE to POST change in peak CMJ displacement.

4.4.7 Body composition

There were main effects of time for changes in both lower-body lean mass (*P* < 0.001) and total lean mass (*P* = 0.006).

4.4.7.1 Lower-body lean mass

Lower-body lean mass (Figure 4.4E) similarly increased from PRE to POST for RT (4.1 ±2.0%; ES; 0.33 ±0.16; *P* = 0.023) and MICT+RT (3.6 ±2.4%; ES; 0.45 ±0.30; *P* = 0.052); however, this change was attenuated for HIT+RT (1.8 ±1.6%; ES; 0.13 ±0.12; *P* = 0.069). Only trivial between-group differences in the PRE to POST change in lower-body lean mass were noted for RT compared with HIT+RT (2.2 ±2.8%; ES, 0.18 ±0.23) and for HIT+RT compared with MICT+RT (1.7 ±3.1%; ES, 0.16 ±0.28).

4.4.7.2 Upper-body lean mass

Changes in upper-body lean mass (Figure 4.4F) between PRE and POST were trivial for both RT (0.4 ±1.9%; ES; 0.02 ±0.19; *P* = 0.719) and HIT+RT (1.4 ±2.0%; ES; 0.13 ±0.17; *P* = 0.198); however, a possibly small increase was noted for MICT+RT (1.8 ±2.9%; ES; 0.17 ±0.28; *P* = 0.325).

4.4.7.3 Total lean mass

Total lean mass (Table 4.3) was not substantially different from PRE to POST for RT (1.6 ±1.4%; ES; 0.12 ±0.10; *P* = 0.102), HIT+RT (1.6 ±1.1%; ES; 0.14 ±0.09; *P* = 0.038) or MICT+RT (2.4 ±2.4%; ES; 0.27 ±0.26; *P* = 0.151).

4.4.7.4 Body fat percentage

Body fat percentage (Table 4.3) was not substantially changed from PRE to POST for RT (-0.6 ±1.0%; ES; -0.08 ±0.17; *P* = 0.372), HIT+RT (-0.2 ±0.9%; ES; -0.03 ±0.15; *P* = 0.659) or MICT+RT (-0.9 ±1.0%; ES; -0.25 ±0.30; *P* = 0.115).

4.4.8 Aerobic capacity

There was a main effect of time for changes in the lactate threshold (*P* = 0.005), and main effects for time (*P* = 0.036) and a time × group interaction (*P* = 0.041) for changes in peak aerobic power.

4.4.8.1 Peak oxygen consumption ($\dot{V}O_{2peak}$)

Absolute peak oxygen consumption ($\dot{V}O_{2peak}$; Table 4.3) was increased from PRE to POST for HIT+RT (5.3 ±2.7%; ES, 0.25 ±0.12; *P* = 0.162) and was possibly increased for MICT+RT (6.1 ±5.0%; ES, 0.27 ±0.22; *P* = 0.103), but unchanged for RT (-0.6 ±6.4%; ES, -0.02 ±0.21; *P* = 0.876). There were no substantial differences in the PRE to POST change in $\dot{V}O_{2peak}$ between HIT+RT and MICT+RT.

4.4.8.2 Lactate threshold (LT)

Lactate threshold (LT; Table 4.3) was increased from PRE to POST for MICT+RT (12.6 ±8.0%; ES, 0.30 ±0.18; *P* = 0.107), but was not substantially different for either HIT+RT (8.3 ±6.5%; ES, 0.20 ±0.15 *P* = 0.054) or RT (7.4 ±9.4%; ES, 0.13 ±0.16; *P* = 0.080). There were no substantial between-group differences in the PRE to POST change in LT.

4.4.8.3 Peak aerobic power (W_{peak})

Peak aerobic power (W_{peak}; Table 4.3) was increased from PRE to POST for HIT+RT (8.8 ±4.1%; ES, 0.31 ±0.14; *P* = 0.010) and MICT+RT (4.9 ±4.8%; ES, 0.19 ±0.18; *P* = 0.096), but unchanged for RT (-2.2 ±6.5%; ES, -0.06 ±0.17; *P* = 0.515). The PRE to POST change in W_{peak} was also greater for HIT+RT compared with RT (11.3 ±8.1%; ES, 0.35 ±0.24), but not MICT+RT (7.3 ±7.8%; ES, 0.24 ±0.25).

Table 4.3 Physical characteristics of participants, exercise performance and body composition data at PRE-, MID- and POST-training for all Study 2 training groups. Data shown are group means ± SD. RT, resistance training; HIT, high-intensity interval training; MICT, moderate-intensity continous training. † = *P* < 0.05 vs. PRE-training; # = *P* < 0.05 vs. HIT+RT, ^ = *P* < 0.05 vs. MICT+RT; ‡ = *P* < 0.05 vs. RT.

	RT			HIT+RT			MICT+RT		
	PRE	MID	POST	PRE	MID	POST	PRE	MID	POST
Physical characteristics									
Age (y)	28.6 ± 6.4	-	-	29.5 ± 2.1	-	-	30.8 ± 7.1	-	-
Height (cm)	182.7 ± 7.6	-	-	181.3 ± 5.8	-	-	183.3 ± 4.2	-	-
Body mass (kg)	86.6 ± 14	-	85.9 ± 14.6	82.6 ± 10.9	-	83.3 ± 11.7	85.5 ± 9.8	-	85.4 ± 8
Maximal strength									
1-RM leg press (kg)	300.5 ± 59.2	350.3 ± 52.2 †	411.8 ± 53.1 †#^	298.9 ± 55.7	340.5 ± 61.7 †	383.0 ± 60.2 †	291.2 ± 68.3	335.1 ± 68.8 †	365.9 ± 59.9 †
1-RM bench press (kg)	69.7 ± 20.8	80 ± 23.3 †#^	83.1 ± 22.0 †	78.4 ± 15.4	85 ± 16 †	90.4 ± 15.3 †	79.3 ± 24.9	84 ± 21.8 †	90 ± 23.7 †
CMJ variables									
Peak force (N)	1847 ± 266	1887 ± 258^	1977 ± 224 †#^	1777 ± 231	1779 ± 242	1784 ± 277	1872 ± 318	1814 ± 310 †	1847 ± 232
Peak power (W)	2835 ± 272	2876 ± 199	3208 ± 468 †	2699 ± 379	2804 ± 361	2799 ± 469	2917 ± 646	2949 ± 471	3065 ± 501
Peak velocity ($m \cdot s^{-1}$)	1.88 ± 0.41	1.90 ± 0.36	2.04 ± 0.34 †	1.80 ± 0.18	1.82 ± 0.18	1.85 ± 0.19	1.83 ± 0.18	1.85 ± 0.13	1.94 ± 0.19 †
Peak displacement (m)	0.48 ± 0.13	0.49 ± 0.12	0.52 ± 0.12	0.43 ± 0.06	0.45 ± 0.05	0.47 ± 0.07	0.48 ± 0.09	0.47 ± 0.05	0.51 ± 0.09

	RT			HIT+RT			MICT+RT		
	PRE	MID	POST	PRE	MID	POST	PRE	MID	POST
Body composition									
Upper-body lean mass (kg)	39.7 ± 5.5	-	39.8 ± 5.1	38.6 ± 3.7	-	39.1 ± 3.5	39.3 ± 3.6	-	39.9 ± 2.9
Lower-body lean mass (kg)	21.2 ± 2.2	-	22.0 ± 1.8 †	21.4 ± 2.5	-	21.9 ± 2.5	21.8 ± 1.5	-	22.5 ± 1.1
Total lean mass (kg)	60.85 ± 7.2	-	61.7 ± 6.5	60.1 ± 6.0	-	60.9 ± 5.5	61.0 ± 4.9	-	62.4 ± 3.7
Body fat (%)	18.2 ± 7.1	-	17.6 ± 6.9	17.0 ± 5.6	-	16.8 ± 5.7	18.8 ± 3.5	-	17.9 ± 3.4
Aerobic capacity									
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	42.2 ± 12.6	41.3 ± 8.9	40.7 ± 9.4	47.3 ± 13.4	47.4 ± 10.2	48.4 ± 10.0	43.4 ± 6.9	47.8 ± 9.7	45.4 ± 6.1
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	3.46 ± 0.78	3.49 ± 0.53	3.41 ± 0.64	3.80 ± 0.75	3.82 ± 0.52	3.96 ± 0.41	3.64 ± 0.38	4.10 ± 0.85 †	3.86 ± 0.29
Lactate threshold (W)	145 ± 48	153 ± 48	155 ± 49	182 ± 53	182 ± 51	196 ± 47	159 ± 55	165 ± 40	174 ± 40
Peak aerobic power (W)	245 ± 56	243 ± 60	239 ± 56	279 ± 55	288 ± 57	301 ± 46 †‡	267 ± 43	269 ± 47	279 ± 38

4.5 Discussion

This is the first investigation to compare the effects of HIT and work-matched MICT on adaptations to maximal strength, CMJ performance, and lean mass when performed concurrently with RT. The main findings of this study were that, compared with RT performed alone, concurrent training incorporating either HIT or work-matched MICT cycling similarly attenuated maximal lower-body strength development and improvements in peak CMJ force and power, while increases in lower-body lean mass were attenuated with concurrent training incorporating HIT, but not MICT.

Since the classic work of Hickson (1980), many studies have reported attenuated increases in maximal strength (Bell et al., 2000; Chtara et al., 2008; Craig et al., 1991; Gergley, 2009; Hickson, 1980; Kraemer et al., 1995), hypertrophy (Bell et al., 2000; Kraemer et al., 1995) and indices of power development (Chtara et al., 2008; Hakkinen et al., 2003; Kraemer et al., 1995) with concurrent training compared with RT performed alone. However, variations in the prescription of specific training variables, including training volume, intensity and modality, as well as participant training status, may influence the degree of interference observed (Fyfe et al., 2014). Elucidating the role of these training variables in mediating the interference effect may therefore inform exercise prescription strategies for minimising interference during periods of concurrent training. Given the efficacy of HIT compared with MICT for improving aerobic capacity (Milanovic et al., 2015) and metabolic health (Tjonna et al., 2008; Wisloff et al., 2007), it was sought to determine whether, on a work-matched basis, endurance training intensity modulates any interference effect to RT adaptations during concurrent training.

Previous studies have observed attenuated maximal strength development following concurrent training incorporating HIT (Chtara et al., 2008; Kraemer et al., 1995), MICT (Craig et al., 1991; Gergley, 2009), or combinations of both (Bell et al., 2000; Hickson, 1980). However, it is unclear whether endurance training intensity might be important for mediating any interference effect to maximal strength development. The major finding of this study was that compared with performing RT alone, both HIT and MICT attenuated maximal lower-body strength to a similar extent, but had no influence on upper-body strength development when performed concurrently with RT. This was contrary to our hypothesis, as it was expected interference to RT

adaptations to be exacerbated in the HIT+RT group. Given that the HIT and MICT protocols employed in the present study were both duration- and work-matched, this observation lends support to the notion that endurance training volume (i.e., total work performed) might be a more critical mediator of interference to maximal strength gain during concurrent training than endurance training intensity (Jones et al., 2013; Wilson et al., 2012). Work by Jones and colleagues (2013) showed that altering the ratio of concurrent training, so that resistance- and endurance-like isokinetic contractions were performed at either a 1:1 or 3:1 weekly frequency ratio, led to compromised strength gain only when resistance and endurance exercise were both performed every session (i.e., with a 1:1 ratio). Moreover, performing maximal-intensity, low-volume, sprint interval cycling (i.e., a modified 20-s Wingate protocol) concurrently with RT does not interfere with maximal strength or lean mass improvements after 12 weeks of training (Cantrell et al., 2014). These observations, together with our present data, suggest that endurance training intensity may not be a critical mediator of interference to maximal strength gain with concurrent training, at least when total work is matched.

The observation of limited interference to maximal upper-body strength gain is in agreement with most (Craig et al., 1991; Hunter et al., 1987; Kraemer et al., 1995), but not all (Hennessy & Watson, 1994), concurrent training studies employing lower-body endurance training modalities, suggesting the mechanisms underlying this interference effect are local rather than systemic (Wilson et al., 2012). One mechanism by which concurrent endurance training may mediate any local interference effect is by compromising the quality of subsequent RT sessions (i.e., residual fatigue from prior endurance exercise) (Fyfe et al., 2014). Endurance exercise induces residual fatigue of the exercised musculature, which persists for at least 6 h post-exercise (Bentley et al., 2000), and is exacerbated after high-intensity interval vs. lower-intensity continuous endurance exercise (de Souza et al., 2007). However, whether the endurance training protocols employed in the present study elicited divergent effects on residual fatigue is unclear, although no negative effects of prior endurance exercise on planned RT intensities or volumes were observed for both the HIT+RT and MICT+RT groups. Another mechanism by which maximal strength may be compromised during concurrent training is via a concomitant attenuation in skeletal muscle hypertrophy, which may contribute to a reduction in force generating capacity. The observation of a similar attenuation to maximal lower-body strength gain in both concurrent training groups, together with the attenuated lean mass gain of the lower body for the HIT+RT

group, suggests the interference to maximal strength gains may have been mediated by non-hypertrophic mechanisms. However, no measures of training-induced changes in markers of muscle activation or neuromuscular fatigue were obtained, these mechanisms remain speculative.

Another aspect of adaptation to RT that may be attenuated during concurrent training is the ability to generate force rapidly (Chtara et al., 2008; Hakkinen et al., 2003; Kraemer et al., 1995), which is critical for power development. Adaptations to power development may be more susceptible to an interference effect during concurrent training compared with interference to maximal strength or hypertrophy (Hakkinen et al., 2003; Wilson et al., 2012). For example, 21 weeks of concurrent training attenuated improvements in isometric rate of force development compared with RT performed alone, with no detectable interference to 1-RM strength or maximal isometric force gains (Hakkinen et al., 2003). Moreover, a meta-analysis (Wilson et al., 2012) identified greater discrepancies between concurrent training and single-mode RT in effect sizes for lower-body power development (0.55 vs. 0.91, respectively) compared with differences in effect sizes for muscle hypertrophy (1.23 vs. 0.85, respectively) or maximal strength (1.76 vs. 1.44, respectively) development. A CMJ protocol was employed as a measure of explosive lower-body jumping performance. Jumping ability is considered an important element of successful athletic performance (Markovic, 2007), and indices of CMJ performance, including peak CMJ force and velocity, but not peak displacement, correlate with 20-m and 30-m sprint times in youth soccer players (Chamari et al., 2004). Compromised improvement in either of these variables may therefore coincide with reduced performance during sport-specific activities such as acceleration and changing of direction.

In agreement with the interference to maximal lower-body strength development, concurrent training incorporating either HIT or MICT similarly attenuated improvements in peak CMJ force and power compared with RT performed alone. This same interference effect was, however, not observed with other CMJ variables, including peak velocity and displacement. These data suggest interference to peak CMJ power with concurrent training was predominantly related to a reduction in peak CMJ force, but not peak velocity, and that these reductions did not translate into compromised peak CMJ displacement. Previous work by Chtara et al. (Chtara et al., 2008) found that performing HIT running concurrently with circuit-style RT attenuated improvements in several CMJ performance variables, including peak CMJ force, peak

CMJ power, and jumping height. However, others have found no interference to vertical jump height improvements with concurrent training incorporating high-intensity running, compared with RT alone (Balabinis et al., 2003). Our data lend support to the notion that concurrent training interferes with RT-induced improvements in peak CMJ force and power, which appears to be primarily related to attenuated improvement in peak CMJ force rather than velocity. Moreover, the attenuation of peak CMJ force and power with concurrent training may be unrelated to the intensity of endurance training employed, at least when compared on a work-matched basis.

Despite our observations of interference to maximal strength gain and improvements in peak CMJ force and power, there was little evidence this could be attributed to between-group differences in muscle mass gain. Previous studies have reported attenuated markers of muscle hypertrophy following concurrent training incorporating combinations of moderate- and high-intensity endurance training (Bell et al., 2000; Kraemer et al., 1995), compared with RT performed alone. However, others have observed no evidence of interference to muscle hypertrophy following lower-intensity, continuous endurance training (Lundberg et al., 2013; McCarthy et al., 2002). Whether the intensity of endurance training employed played a role in any interference to muscle hypertrophy development is therefore unclear. While similar increases in lower-body lean mass were noted for the MICT+RT group compared with RT performed alone, this improvement was attenuated for the HIT+RT group. Despite these differences, only trivial effects (ES, 0.18 and 0.16 for HIT+RT and MICT+RT compared with RT, respectively) were observed for between-group differences in the training-induced change in lower-body lean mass. Our data suggests that, on a work-matched basis, performing higher-intensity endurance training concurrently with RT may compromise lean mass gain, which is specific to the musculature involved in both exercise modalities. Regardless, any small effect of concurrent training on lean mass responses were not reflected in the training-induced changes in both maximal strength and CMJ variables, suggesting that interference to these measures may be mediated by non-hypertrophic (and potentially neural) mechanisms.

It is possible the degree of RT-induced hypertrophy in the present study may have affected the likelihood of detecting clear between-group effects for interference to muscle hypertrophy with concurrent training. Whole-body lean mass gains observed in the present study (700-1400 g) is, however, similar to that reported in other studies utilizing DXA as a measure of lean mass gain (300-2300 g) following 6 (Candow et al.,

2006) or 12 (Rakobowchuk et al., 2005) weeks of RT in the absence of targeted protein supplementation. Nevertheless, between-study differences in RT prescription may impact upon the degree of training-induced lean mass gain. The RT program in the present study was designed primarily to elicit improvements in maximal strength, with a linear progression from high-volume, moderate-intensity RT, to low-volume, high-intensity RT. While this increase in relative exercise intensity was likely favourable for maximizing strength gain, the reduced volumes associated with higher training intensities may be suboptimal for maximizing skeletal muscle hypertrophy (Burd et al., 2010). In addition to training prescription, dietary protein supplementation may also further increase lean mass gain consequent to RT (Phillips & Van Loon, 2011). As the participants in this study were not provided with protein supplementation in the present study, this may have also limited the degree of training-induced muscle hypertrophy, and should be a consideration for future studies. Indeed, the self-reported protein intakes of the participants in the present study (1.11-1.29 $g \cdot kg^{-1} \cdot day^{-1}$) may have been lower than optimal for promoting hypertrophy (1.3-1.8 $g \cdot kg^{-1} \cdot day^{-1}$) (Phillips & Van Loon, 2011). Nevertheless, given average daily protein intake was similar between training groups at baseline, and participants were asked to maintain habitual dietary practices during the intervention period, it is anticipated that between-group differences in training outcomes were not mediated by differences in amino acid availability.

In addition to quantifying internal training load for training sessions performed as part of the training intervention (i.e., prescribed training load), a custom, web-based training diary was used to also quantify internal training load for all training sessions participants completed outside of the study during the intervention period (i.e., non-prescribed training load). This was employed primarily as a surrogate measure for external training volume for all training groups, which may have influenced adaptation consequent to our training intervention. As expected, total training load responses were substantially higher for both concurrent training groups compared with the RT group. Using this approach, it was also found that the non-prescribed internal training load was higher for the HIT+RT group compared with both the MICT+RT and RT groups, which contributed to a higher total study combined training load for HIT+RT compared with MICT+RT. This suggests total training volume may actually have been higher for the HIT+RT group, despite our HIT intervention being work-matched with MICT. Given the discrepancy in total training load between the HIT+RT and MICT+RT groups, it is difficult to deduce whether differences in outcomes such as lean mass changes are

mediated by endurance training intensity or total training volume *per se*. Moreover, as lower-body 1-RM strength was similarly attenuated for the HIT+RT and MICT+RT groups compared with the RT group, this potentially suggests a superiority of HIT compared with MICT for promoting maximal strength gain during concurrent training, when compared on an internal training load-matched basis.

There is accumulating evidence for the greater efficacy of HIT for improving $\dot{V}O_{2peak}$ compared with MICT (Milanovic et al., 2015). However, it has also been shown that improvements in $\dot{V}O_{2peak}$ and the lactate threshold are similar after work-matched HIT and MICT (Edge et al., 2006). Our results suggest that, on a work-matched basis and when performed concurrently with RT, HIT and MICT similarly increase $\dot{V}O_{2peak}$, the LT and W_{peak}, although HIT was more effective in improving the W_{peak} compared with MICT. Improvements in these parameters were similar despite internal training load being substantially higher for HIT compared with MICT. These observations question the potency of HIT compared with traditional MICT for improving markers of aerobic capacity during concurrent training, although direct measures of endurance performance (e.g., distance- or work-based cycling time trial) were not evaluated. The present data also suggest that these divergent exercise intensities do not differentially modulate interference to maximal strength gain, at least on a work-matched basis, and after eight weeks of training in recreationally-active males. It remains to be determined whether more prolonged periods of concurrent training, incorporating either HIT or MICT as the predominant endurance training modality, are associated with divergent effects on interference to RT adaptations.

The potential for individual responses to concurrent training, and subsequently interference to RT adaptations, should also be considered in the context of the present data. It is clear from the variability in training-induced changes in performance measures (Figure 4.4) that there indeed appears to be responders and non-responders to the training intervention, supporting previous observations following both endurance (Bouchard & Rankinen, 2001) and RT (Hubal et al., 2005). It is recognised, however, that appropriate quantification of individual responses to controlled trials requires a large sample size or averaging of repeated measurements to compensate for a large error of measurement (Hopkins, 2015), both of which are unfortunately lacking in the present investigation. Future studies should, where possible, incorporate study designs with increased sample sizes and repeated measurements of performance and morphological

measures, which will subsequently improve the ability of the future studies to make clear inferences about individual responses to training.

4.6 Conclusions

This is the first report of the effects of incorporating either HIT or work-matched MICT into a concurrent training program on adaptations of maximal strength, CMJ performance, aerobic capacity and body composition compared with performing RT alone. In summary, it was demonstrated that HIT and MICT similarly attenuated the RT-induced increase in maximal lower-, but not upper-body, strength, as well as increases in peak CMJ force and power. These observations suggest that endurance training volume may be a more critical mediator of interference to maximal strength gain rather than training intensity. Training-induced increases in lower-body lean mass were attenuated for the HIT+RT group relative to MICT+RT and RT, although the magnitude of between-group differences in lean mass gain were trivial. Total training load was higher for the HIT+RT group compared with the MICT+RT group, due primarily to a higher non-prescribed training load, which may have contributed to the attenuation of the lower-body lean mass gain for the HIT+RT group. Future work should further explore the role of endurance training volume in the interference effect, and whether low-volume HIT may confer benefits by minimising interference when compared with higher volume HIT or MICT during periods of concurrent training.

Chapter 5. Skeletal muscle ribosome biogenesis and mTORC1 signalling responses with concurrent training incorporating high-intensity interval or work-matched continuous endurance training

Adapted from: Fyfe, J.J., Bartlett, J.D., Hanson, E.D., Anderson, M.J., Garnham, A.P., Bishop, D.J., & Stepto, N.K. (2016). Ribosome biogenesis adaptation and mTORC1 signalling in human skeletal muscle following concurrent training compared with single-mode resistance training. *Journal of Applied Physiology*. (In preparation).

5.1 Linking statement

Chapter 4 (Study 2) investigated whether RT adaptations are altered with concurrent training, incorporating either HIT or work-matched MICT, compared with RT performed alone. These data extend current knowledge by suggesting interference to maximal strength gain is not differentially modulated with concurrent training incorporating either HIT or MICT, at least on a work-matched basis, and lean mass gain appears compromised with concurrent training incorporating HIT, but not MICT. It is unclear, however, whether these responses are associated with any interference to the molecular pathways regulating skeletal muscle growth. Chapter 3 (Study 1) of this thesis demonstrated mTORC1 signalling was not compromised with a single bout of concurrent exercise compared with RE performed alone, and HIT alone was a potent stimulus for inducing mTORC1 signalling compared with both MICT and RT. However, considering the limitations of measuring early post-exercise molecular responses in skeletal muscle of relatively training-unaccustomed individuals, further work is required to determine the effects of training on these responses in a basal state, and whether these responses are differentially regulated in skeletal muscle of training-accustomed individuals. Emerging evidence of the role of ribosome biogenesis in promoting muscle growth has provided a novel area for investigation in the context of concurrent training. Observations that markers of ribosome biogenesis are increased in human skeletal muscle following RT, and reduced in models of attenuated muscle mass such as in ageing and with chronic inflammation, suggest ribosome biogenesis is tightly coupled to changes in skeletal muscle mass. The aim of the following chapter was to examine, for the first time, the modulation of ribosome biogenesis adaptation and mTORC1 signalling in human skeletal muscle both at rest after eight weeks of concurrent training vs. single-mode RT, and following group-specific single exercise bouts performed in a training-accustomed state.

5.2 Introduction

Simultaneously incorporating both resistance (RT) and endurance training into a periodised training program, termed concurrent training (Leveritt et al., 1999), can attenuate RT adaptations such as muscle hypertrophy compared with RT performed alone (Bell et al., 2000; Hickson, 1980; Kraemer et al., 1995). This is potentially mediated by an altered balance between post-exercise skeletal muscle protein synthesis (MPS) and breakdown, subsequently attenuating lean mass accretion. The mechanistic target of rapamycin complex 1 (mTORC1) is a key mediator of load-induced increases in MPS and subsequently muscle hypertrophy (Bodine et al., 2001b; Drummond et al., 2009). The activity of mTORC1 is antagonised by activation of the 5' adenosine monophosphate-activated protein kinase (AMPK) acting to restore perturbations in cellular energy balance by inhibiting anabolic cellular processes and stimulating catabolism (Kimball, 2006). In rodent skeletal muscle, low-frequency electrical stimulation mimicking endurance exercise-like contractions promotes AMPK activation and inhibition of mTORC1 signalling (Atherton et al., 2005). Subsequent work in humans (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015) has focused on the hypothesis that attenuated muscle hypertrophy with concurrent training (Bell et al., 2000; Kraemer et al., 1995; Wilson et al., 2012) may be explained by AMPK-mediated inhibition of the mTORC1 pathway. Several studies have, however, demonstrated that single bouts of concurrent exercise do not compromise either mTORC1 signalling or rates of MPS (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Donges et al., 2012; Pugh et al., 2015), and may even potentiate these responses (Lundberg et al., 2012) compared with resistance exercise (RE) performed alone. Transient changes in translational efficiency after single bouts of concurrent exercise, as indexed by mTORC1 signalling or rates of MPS in skeletal muscle, therefore do not appear to explain interference to muscle hypertrophy following longer-term concurrent training.

Rates of cellular protein synthesis are determined not only by transient changes in translational efficiency (i.e., rates of protein synthesis per ribosome), but also by translational capacity (i.e., amount of translational machinery per unit of tissue, including ribosomal content) (Chaillou et al., 2014). Ribosomes are supramolecular ribonucleoprotein complexes functioning at the heart of the translational machinery to

convert mRNA transcripts into protein (Chaillou et al., 2014), and ribosomal content dictates the upper limit of cellular protein synthesis (Iadevaia et al., 2014). Early rises in protein synthesis in response to anabolic stimuli (e.g., a single bout of RE) are generally thought to be mediated by transient activation of existing translational machinery, whereas chronic anabolic stimuli (e.g., weeks to months of RE training) induce an increases in total translational capacity via ribosome biogenesis (Chaillou et al., 2014).

Ribosome biogenesis is a complex, well-orchestrated process involving transcription of the polycistrionic 45S rRNA (ribosomal RNA) precursor (45S pre-rRNA), processing of the 45S pre-rRNA into several smaller rRNAs (18S, 5.8S and 28S rRNAs), assembly of these rRNAs and other ribosomal proteins into ribosomal subunits (40S and 60S), and nuclear export of these ribosomal subunits into the cytoplasm (Chaillou et al., 2014; Thomson et al., 2013). The synthesis of the key components of the ribosomal subunits is achieved via the coordinated actions of three RNA polymerases (RNA Pol-I, -II, and -III). The RNA Pol-I is responsible for the transcription of the 45S pre-rRNA in the nucleolus, which is considered the rate-limiting step in ribosome biogenesis (Moss & Stefanovsky, 1995). The 45S pre-rRNA is subsequently cleaved into the 18S, 5.8S and 28S rRNAs, which undergo post-transcriptional modifications via interactions with small nuclear ribonucleoproteins and several protein processing factors. The RNA Pol-II is responsible for the transcription of ribosomal protein-encoding genes, whereas RNA Pol-III mediates the nucleoplasmic transcription of 5S rRNA and tRNAs (transfer RNAs) (Thomson et al., 2013).

As well as controlling translational efficiency, the mTORC1 is a key mediator of ribosome biogenesis by regulating transcription factors for genes encoding RNA Pol-I and -III (Iadevaia et al., 2014). The transcription of rDNA by RNA Pol-I requires the transcription factor SL-1 (selectivity factor-1), a component of which is TIF-1A (transcription initiation factor 1A; also known as RRN5), as well as other regulatory factors including POLR1B (polymerase [RNA] 1 polypeptide B). Inhibition of mTORC1 by rapamycin inactivates TIF-1A, which impairs the transcription of the 45S pre-rRNA by RNA Pol-I (Mayer et al., 2004). Inhibition of mTORC1 also inactivates UBF (upstream binding factor) (Hannan et al., 2003), a transcription factor also associated with SL-1, while the key mTORC1 substrate p70S6K1 promotes UBF activation and RNA Pol-I-mediated rDNA transcription (Hannan et al., 2003). As well as regulation by mTORC1 signalling, the cyclins (including cyclin-D1) and cyclin-dependent kinases (CDKs) can also regulate UBF via phosphorylation on Ser388 and

Ser484, which are required for UBF activity (Voit & Grummt, 2001; Voit et al., 1999). In addition to regulation of RNA Pol-1, mTORC1 also associates with a number of RNA Pol-III genes that synthesise 5.8S rRNA and tRNA (Kantidakis et al., 2010).

Studies in both human (Figueiredo et al., 2015; Nader et al., 2014; Stec et al., 2015) and rodent (Adams et al., 2002; Chaillou et al., 2012; Chaillou et al., 2013; Goodman et al., 2011a; Miyazaki et al., 2011; von Walden et al., 2012) skeletal muscle suggest ribosome biogenesis, as indexed by increases in total RNA content (>85% of which comprises rRNA) (Chaillou et al., 2014), and increased mRNA expression of several RNA Pol-I regulatory factors, including UBF, cyclin D1 and TIF-1A, occurs concomitantly with muscle hypertrophy. In addition, attenuated rodent skeletal muscle hypertrophy with ageing (Kirby et al., 2015; Stec et al., 2015) and rapamycin treatment (Goodman et al., 2011a) is associated with reduced markers of ribosome biogenesis, suggesting translational capacity is closely linked to the modulation of skeletal muscle mass. Despite the links between skeletal muscle hypertrophy and ribosome biogenesis (Chaillou et al., 2014; Figueiredo et al., 2015; Nader et al., 2014), studies investigating molecular interference following concurrent exercise in human skeletal muscle have only measured transient (<6 h) post-exercise changes in translational efficiency and MPS (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015). No studies have investigated changes in ribosome biogenesis either after single bouts of concurrent exercise or following periods of concurrent training. Whether attenuated muscle hypertrophy following concurrent training could be explained, at least in part, by attenuated ribosome biogenesis is unknown.

The aim of this study to investigate changes in markers of ribosome biogenesis and mTORC1 signalling after eight weeks of concurrent training compared with RT undertaken alone. A secondary aim was to determine the potential role of endurance training intensity in modulating skeletal muscle ribosome biogenesis adaptation to concurrent training, by directly comparing the incorporation of either high-intensity interval training (HIT) or work-matched moderate-intensity continuous training (MICT). The induction of these responses in skeletal muscle was also investigated following a single exercise bout performed post-training. It was hypothesised that compared with RT alone, concurrent training would attenuate the expression of markers of ribosome biogenesis, but not mTORC1 signalling, both at rest post-training and

following a single exercise bout performed in a training-accustomed state. Based on observations gleaned from Chapter 4, it was further hypothesised that concurrent training incorporating HIT would preferentially attenuate training-induced skeletal muscle hypertrophy relative to RT alone, and this would be associated with a likewise attenuation in markers of skeletal muscle ribosome biogenesis.

5.3 Methodology

5.3.1 Participants

Details of the participants for this study are identical to those presented in Chapter 4 (section 4.3.1). After being fully informed of study procedures (Appendix B) and screening for possible exclusion criteria (Appendices C and F), participants provided written informed consent (Appendix E). All procedures were approved by the Victoria University Human Research Ethics Committee.

5.3.2 Study overview

The procedures performed in this study are described in detail in Chapter 4; however, these are briefly summarised as follows. The study employed a repeated-measures, parallel-group design (Figure 5.1A). After preliminary testing for maximal (one-repetition maximum [1-RM]) strength, aerobic capacity ($\dot{V}O_{2peak}$, the lactate threshold [LT] and peak aerobic power [W_{peak}]), and body composition (dual-energy x-ray absorptiometry [DXA]), participants were ranked by baseline 1-RM leg press strength and randomly allocated to one of three training groups. Training groups consisted of 1) high-intensity interval training (HIT) cycling combined with RT (HIT+RT group, n = 8), 2) moderate-intensity continuous training (MICT) cycling combined with RT (MICT+RT group, n = 7) and 3) RT performed alone (RT group, n = 8). After preliminary testing, participants completed 8 weeks of group-specific training performed three times per week. Two or three days after completing the post-training 1-RM strength testing, participants underwent a group-specific single-bout exercise trial (Figure 5.1B) to measure early post-exercise molecular responses in skeletal muscle

related to both translational efficiency and translational capacity in a training-accustomed state.

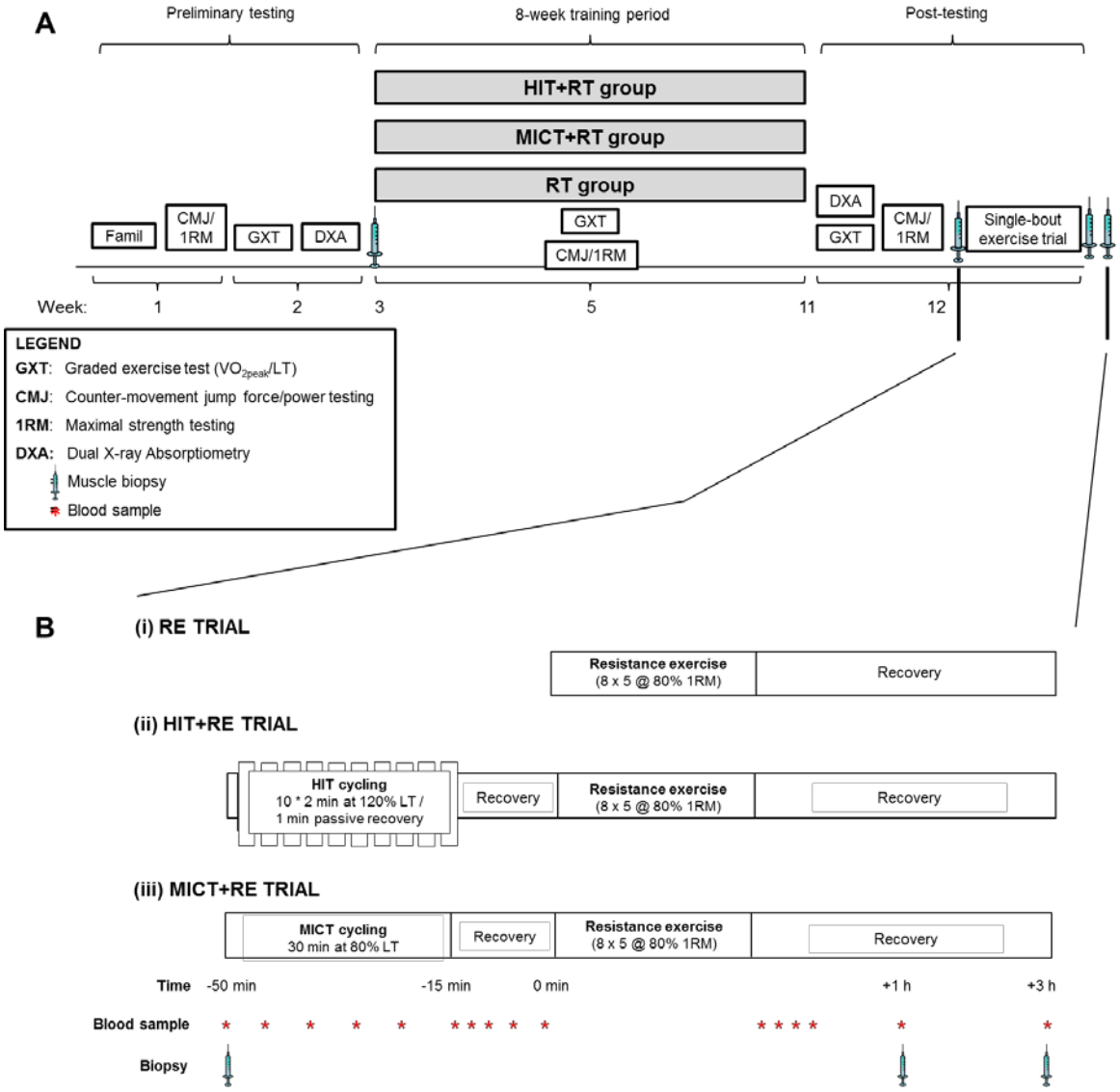

Figure 5.1 Study overview (A) and single-bout exercise trial timelines (B) employed for Study 2. Participants completed 8 weeks of either resistance training (RT) alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT). For the post-training single-bout exercise trials (B), participants completed either the RE protocol either alone (i) or after a 15-min recovery from a single bout of either HIT (ii) or work-matched MICT (iii) cycling. Muscle biopsies were obtained from the vastus lateralis at rest before training and immediately before exercise in the single-bout exercise trial, and 1 h and 3 h after completion of RE.

5.3.2.1 Training intervention

The training intervention performed for this study is described in detail in Chapter 4 (see section 4.3.4). Briefly, participants began the 8-week training intervention 3-5 days after completion of preliminary testing (see section 4.3.3). All training groups performed an identical RT program on non-consecutive days (typically Monday, Wednesday, and Friday), with the HIT+RT and MICT+RT groups completing the corresponding form of endurance exercise 10 min prior to commencing each RT session. See Table 4.1 and Table 4.2 for details of the progression of both endurance and reisstance training throughout the 8-week intervention.

5.3.2.2 Post-training single-bout exercise trial

Two or three days after completion of the training intervention and post-testing, participants perfomed a single-bout exercise trial (Figure 5.1B) to measure early post-exercise skeletal muscle responses in a training-accustomed state. Participants reported to the laboratory after an overnight (~8-10 h) fast. After resting quietly for ~15 min upon arrival at the laboratory, a venous cathether was inserted into an anticubital forearm vein and a resting blood sample was obtained. A resting muscle biopsy was then taken from the *vastus lateralis* muscle (described subsequently). Participants in the RT group then completed a standardised RT protocol (8 x 5 leg press repetitions at 80% of the post-training 1RM, 3 min recovery between sets) alone, with participants in the HIT+RT and MICT+RT groups preceding the standardised RT bout 15 min prior with either HIT (10 x 2-min intervals at 140% of the post-training LT, 1 min passive recovery between intervals) or work- and duration-matched MICT cycling (30 min at 93.3% post-training LT), respectively. Each cycling bout was performed after a standardised warm-up ride at 75 W for 5 min. After completion of RT, participants rested quietly in the laboratory and additional biopsies were obtained after 1 (+1 h) and 3 h (+3 h) of recovery. Biopsies were obtained from separate incision sites in a proximal-to-distal fashion on the same leg as the pre-training biopsy. Venous blood samples were also obtained at regular intervals during cycling and following recovery from both cycling and RT (Figure 5.1B).

5.3.2.3 Muscle sampling

Immediately prior to the first training session (i.e., at least 3 days after completion of preliminary testing), a resting muscle biopsy was obtained from the *vastus lateralis* using the percutaneous needle biopsy technique (Bergstrom, 1962) modified with suction (Evans et al., 1982). Three additional biopsies were obtained during the single-bout exercise trial performed post-training. After administration of local anaesthesia (1% Xylocaine), a small incision (~8 mm in length) was made through the skin, subcutaneous tissue and fascia overlying the *vastus lateralis* muscle for each subsequent biopsy. A 6 mm Bergström needle was then inserted into the muscle and a small portion of muscle tissue (~50-400 mg) removed. All biopsies were obtained from the same leg for each participant, and in a proximal-to-distal fashion. Muscle samples were blotted on filter paper to remove excess blood, immediately frozen in liquid nitrogen, and stored at –80°C until subsequent analysis. A small portion of each biopsy sample (~20 mg) was embedded in Tissue-Tek (Sakura, Finetek, NL), frozen in liquid nitrogen-cooled isopentane, and stored at –80°C for subsequent immunofluorescence analysis.

5.3.3 Western blotting

Approximately 5 mg of frozen muscle tissue was homogenised in lysis buffer (0.125M Tris-HCl, 4% SDS, 10% Glycerol, 10mM EGTA, 0.1M DTT, 1% protease/phosphatase inhibitor cocktail), left for 1 h at room temperature, and then stored overnight at -80°C. The following morning, samples were thawed and the protein concentration determined (Red 660 Protein Assay Kit, G-Biosciences, St. Louis, MO). Bromophenol blue (0.1%) was then added to each sample, which were then stored at -80°C until subsequent analysis. Proteins (8 µg) were separated by SDS-PAGE using 6-12% acrylamide pre-cast gels (TGX Stain Free, Bio-Rad laboratories, Hercules, CA) in 1× running buffer (25 mM Tris, 192 mM Glycine, 0.1% SDS), and transferred to polyvinylidine fluoride (PVDF) membranes (Bio-Rad laboratories, Hercules, CA) using a semi-dry transfer system (Trans Blot Turbo, Bio-Rad laboratories, Hercules, CA) for 7 min at 25 V. After transfer, membranes were blocked with 5% skim milk in 1×TBST (200 mM Tris, 1.5 M NaCl, 0.05% Tween 20) for 1 h at room temperature, washed with 1×TBST (5×5 min), and incubated with primary antibody solution (5% BSA [bovine serum albumin], 0.05% Na Azide in 1×TBST) overnight at 4°C. Primary antibodies for

phosphorylated (p-) p-mTOR^{Ser2448} (1:1000; #5536), mTOR (1:1000), p-p70S6K1^{Thr389} (1:1000; #9234), p70S6K1 (1:1000), p-4E-BP1$^{Thr37/46}$ (1:1000; #2855), 4E-BP1 (1:1000; #9452), p-AMPK^{Thr172} (1:1000; #2535), AMPK (1:1000; #2532), p-rps6$^{Ser235/236}$ (1:750; #4856), rps6 (1:1000; #2217), p-GSK-3α/β$^{Ser21/9}$ (1:1000; #9331), GSK-3β (1:1000; #9315), p-eEF2^{Thr56} (1:1000; #2331), p-ACC^{Ser79} (1:1000; #3661) and ACC (1:1000; #3662) were from Cell Signalling Technology (Danvers, MA), p-UBF^{Ser388} (1:000; sc-21637-R), UBF (1:000; sc-9131) and cyclin D1 (1:1000; sc-450) were from Santa Cruz Biotechnology (Dallas, TX), and p-RRN3 (TIF-1A)^{Ser649} (1:000; ab138651) and TIF-1A (1:000; ab70560) were from Abcam (Cambridge, UK). The following morning, membranes were washed again with 1×TBST and incubated with a secondary antibody (Perkin Elmer, Waltham, MA, #NEF812001EA; 1:50000 or 1:100000 in 5% skim milk and 1×TBST) for 1 h at room temperature. After washing again with 1×TBST, proteins were detected with chemiluminescence (SuperSignal^{TM} West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific, Waltham, MA) and quantified via densitometry (Image Lab 5.0, Bio-Rad laboratories, Hercules, CA). All sample timepoints for each participant were run on the same gel and normalised to both an internal pooled sample present on each gel and the total protein content of each lane using a stain-free imaging system (Chemi Doc^{TM} MP, Bio-Rad laboratories, Hercules, CA). Phosphorylated proteins were then expressed relative to the total amount of each respective protein.

5.3.4 Real-time quantitative PCR (qPCR)

5.3.4.1 RNA extraction

Total RNA (1145 ± 740 ng; mean ± SD) was extracted from approximately 25 mg of muscle tissue using TRI Reagent® (Sigma Aldrich, St. Louis, MO) according to the manufacturer's protocol. Muscle samples were firstly homogenised in 500 μL of TRI Reagent® using a Tissue Lyser II and 5 mm stainless steel beads (Qiagen, Venlo, Limburg, Netherlands) for 120 s at 30 Hz. After resting for 5 min on ice, 50 μL of 1-bromo-3-chloropropane (BCP) was added to the tube, inverted for 30 s to mix, and then rested for 10 min at room temperature. The homogenate was then centrifuged for 15 min at 13,000 rpm and the upper transparent phase transferred to another tube. Isopropanol (400 μL) was added to the tube, inverted briefly to mix, and stored overnight at -20°C to precipitate the RNA. After overnight incubation, the solution was

centrifuged for 60 min at 13,000 rpm to pellet the RNA. The RNA pellet was washed twice by centrifugation in 75% ethanol/nuclease-free water (NFW) for 15 min at 13,000 rpm, allowed to air-dry, and then dissolved in 15 μL of NFW (Ambion Inc., Austin, TX). The quantity and quality of RNA was subsequently determined using a spectrophotometer (BioPhotometer, Eppendorf, Hamburg, Germany). The purity of RNA was assessed using the ratio between the absorbance at 260 nm and absorbance at 280 nm (mean ± SD; 2.37 ± 0.43), and the ratio between the absorbance at 260 nm and absorbance at 230 nm (1.71 ± 0.42). The total muscle RNA concentration was calculated based on the total RNA yield relative to the wet weight of the muscle sample.

5.3.4.2 Reverse transcription

For mRNA analysis, first-strand cDNA was generated from 1 μg RNA in 20 μL reaction buffer using the iScript® cDNA synthesis kit (Bio-Rad laboratories, Hercules, CA) according to manufacturer's protocol, with each reaction comprising 4 μL 5× iScript reaction mix, 1 μL iScript Reverse Transcriptase, 5 μL NFW and 10 μL of RNA sample (100 ng/μL). Reverse transcription was then performed with the following conditions: 5 min at 25°C to anneal primers, 30 min at 42°C for the extension phase, and 5 min at 85°C. Following reverse transcription, samples were DNase-treated (Life Technologies, Carlsbad, CA) and cDNA was stored at -20°C until further analysis.

5.3.4.3 Real-time quantitative PCR (qPCR)

Real-time PCR was performed using a Realplex^2 PCR system (Eppendorf, Hamburg, Germany) to measure mRNA levels of MuRF-1 (muscle RING-finger 1), Atrogin-1 (muscle atrophy f-box), FoxO1 (forkhead box-O1), PGC-1α (peroxisome proliferator-activated gamma receptor co-activator-1 alpha), UBF, TIF-1A, cyclin D1, POLR1B, and commonly used reference genes GAPDH (glyceraldehyde 3-phosphate dehydrogenase), cyclophilin (also known as peptidyl-prolylcis-trans isomerase), β2M (beta-2 microglobulin) and TBP (TATA binding protein). Target rRNAs were the mature ribosome species 5.8S, 18S and 28S. Since primers specific for these mature rRNA sequences will also amplify pre-RNA transcripts (i.e., the 45S pre-rRNA), we used specifically designed primers (QIAGEN, Venlo, Limburg, The Netherlands) to distinguish between mature rRNA species and those still bound to the 45S pre-rRNA transcript, as previously described (Figueiredo et al., 2015). Briefly, primers were

designed specifically for pre-rRNA sequences spanning the 5'end external/internal transcribed spacer regions (ETS and ITS, respectively) of the 45S pre-RNA transcript and the internal regions of mature rRNA sequences (i.e., 18S-ETS, 5.8S-ITS, and 28S-ETS). For clarity, primers amplifying the mature rRNA transcripts are henceforth designated as 'mature' transcripts (e.g., 18S rRNA [mature]), as opposed to those primers amplifying rRNA sequences bound to the 45S rRNA precursor, henceforth designated as 'span' transcripts (e.g., 18S rRNA [span]). A specific primer for the initial region of the 5' end of the 45S pre-rRNA transcript was used to measure 45S pre-rRNA expression levels (Figueiredo et al., 2015). Standard and melting curves were performed for all primers to ensure both single-product and amplification efficiency. Details for all primers used are provided in Table 5.1 (mRNA) and Table 5.2 (rRNA).

Table 5.1 Details of PCR primers used for Study 2 mRNA analysis

Gene	Forward sequence	Reverse sequence	NCBI reference sequence
MuRF-1	5'-CCTGAGAGCCATTGACTTTGG-3'	5'-CTTCCCTTCTGTGGACTCTTCCT-3'	NM_032588.3
Atrogin-1	5'-GCAGCTGAACAACATTCAGATCAC-3'	5'-CAGCCTCTGCATGATGTTCAGT-3'	NM_058229.3
Fox-O1	5'-TTGTTACATAGTCAGCTTG-3'	5'-TCACTTTCCTGCCCAACCAG-3'	NM_002015.3
PGC-1α	5'-GGCAGAAGGCAATTGAAGAG-3'	5'-TCAAAACGGTCCCTCAGTTC-3'	NM_013261.3
UBF	5'-CCTGGGGAAGCAGTGGTCTC-3	5'-CCCTCCTCACTGATGTTCAGC-3	XM_006722059.2
TIF-1A	5'-GTTCGGTTTGGTGGAACTGTG-3	5'-TCTGGTCATCCTTTATGTCTGG-3	XM_005255377.3
Cyclin D1	5'-GCTGCGAAGTGGAAACCATC-3	5'-CCTCCTTCTGCACACATTTGAA-3	NM_053056.2
POLR1B	5'-GCTACTGGGAATCTGCGTTCT-3	5'-CAGCGGAAATGGGAGAGGTA-3	NM_019014.5
TBP	5'-CAGTGACCCAGCAGCATCACT-3'	5'-AGGCCAAGCCCTGAGCGTAA-3'	M55654.1
Cyclophillin	5'-GTCAACCCCACCGTGTTCTTC-3'	5'-TTTCTGCTGTCTTTGGGACCTTG-3'	XM_011508410.1
GAPDH	5'-AAAGCCTGCCGGTGACTAAC-3'	5'-CGCCCAATACGACCAAATCAGA-3'	NM_001256799.2
β2M	5'-TGCTGTCTCCATGTTTGATGTATCT-3'	5'-TCTCTGCTCCCCACCTCTAAGT-3'	NM_004048.2

MuRF-1, muscle RING-finger 1; Fox-O1, forkhead box-O1; PGC-1α, peroxisome proliferator activated receptor gamma co-activator 1 alpha; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; UBF, upstream binding factor; TIF-1A, RRN3 polymerase 1 transcription factor; POLR1B, polymerase (RNA) 1 polypeptide B; TBP, TATA binding protein; β2M, beta-2 microglobulin.

Table 5.2 Details of PCR primers used for Study 2 rRNA analysis

Target	Catalogue number
45S pre-rRNA	PPH82089A
5.8S rRNA (mature)	PPH82091A
18S rRNA (mature)	PPH71602A
28S rRNA (mature)	PPH82090A
5.8S-ITS (span)	PPH82111A
18S-ETS (span)	PPH82110A
28S-ITS (span)	PPH82112A

Each PCR reaction was performed in duplicate using a robotic pipetting machine (EpMotion 2100, Eppendorf, Hamburg, Germany) in a final reaction volume of 10 µL containing 5.0 µL 2× SYBR green (Bio-Rad Laboratories, Hercules, CA), 0.6 µL PCR primers (diluted to 15 µM; Sigma Aldrich, St. Louis, MO), 0.4 µL NFW and 4 µL cDNA sample (diluted to 5 ng/µL). Conditions for the PCR reactions were: 3 min at 95°C, 40 cycles of 15 sec at 95°C/1 min at 60°C, one cycle of 15 sec at 95°C/15 sec at 60°C, and a ramp for 20 min to 95°C. Each plate was briefly centrifuged before loading into the PCR machine. To compensate for variations in input RNA amounts and efficiency of the reverse transcription, mRNA data were quantified using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) and normalised to the geometric mean (Vandesompele et al., 2002) of the three most stable housekeeping genes analysed (cyclophillin, β2M and TBP), determined as previously described (Mane et al., 2008).

5.3.5 Immunohistochemistry

Muscle cross-sections (10 µM) were cut at -20°C using a cryostat (Microm HM 550, Thermo Fisher Scientific, Waltham, MA), mounted on uncoated glass slides, and air-dried for 20 min at room temperature. Sections were then rinsed briefly with 1×PBS (0.1M; Sigma Aldrich, St Louis, MO), fixed with cold paraformaldehyde (4% in 1×PBS) for 10 min at room temperature, rinsed three times with 1×PBS, incubated in 0.5% TritonX in 1×PBS for 5 min at room temperature, rinsed again three times with 1×PBS, and then blocked for 1 h at room temperature in a 3% BSA solution in 1×PBS. After blocking, sections were then incubated with a primary antibody for myosin heavy chain type I (A4.840, Developmental Studies Hybridoma Bank, University of Iowa, IA), diluted 1:25 in 3% BSA/PBS overnight at 4°C. The following morning, sections were washed four times in 1×PBS for 10 min each, before incubating with a secondary

antibody (Alexa Fluor® 488 conjugate Goat anti-mouse IgM, cat. no. A-21042, Thermo Fisher Scientific, Waltham, MA) diluted 1:200 in 3% BSA/PBS for 2 h at room temperature. Sections were again washed four times in 1×PBS for 10 min each, before incubation with Wheat Germ Agglutinin (WGA) (Alexa Fluor® 594 Conjugate; cat. no. W11262, Thermo Fisher Scientific, Waltham, MA), diluted to 1:100 in 1×PBS (from a 1.25 mg/mL stock solution), for 15 min at room temperature. Sections were washed again 4 times with 1×PBS for 3 min each, blotted dry with a Kim-Wipe, and Flouroshield^{TM} (cat. no. F6182; Sigma Aldrich, St Louis, MO) added to each section before the coverslip was mounted. Stained muscle sections were air-dried for ~2 h and viewed with an Olympus BX51 microscope coupled with an Olympus DP72 camera for flourescence detection (Olympus, Shinjuku, Japan). Images were captured with a 10× objective and analysed using Image Pro Premier software (version 9.1; Media Cybernetics, Rockville, MD). Analysis was completed by an investigator blinded to all groups and time points. For each subject, muscle fibre CSA was determined for both type I and type II muscle fibres. For the RT, HIT+RT and MICT+RT groups, a total of 107 ± 61, 112 ± 67, and 84 ± 73 (mean ± SD) type I fibres and 154 ± 72, 136 ± 80, and 144 ± 76 (mean ± SD) type II fibres were included for analysis, respectively.

5.3.6 Statistical analyses

The effect of training group on outcomes was evaluated via a two-way (time × group) analysis of variance with repeated-measures (RM-ANOVA) (SPSS, Version 21, IBM Corporation, New York, NY). Pearson's product moment correlations were used to determine the strength of relationship between dependent variables (SPSS, Version 21, IBM Corporation, New York, NY). Molecular (protein signalling, mRNA and rRNA expression) data were log-transformed before analysis to reduce non-uniformity of error (Hopkins et al., 2009). To quantify the magnitude of within- and between group differences in dependent variables, a magnitude-based approach to inferences using the standardised difference (effect size, ES) was used as previously described (Hopkins et al., 2009). The magnitude of effects were defined according to thresholds suggested by Hopkins (Hopkins et al., 2009), whereby <0.2 = trivial, 0.2-0.6 = small, 0.6-1.2 = moderate, 1.2-2.0 = large, 2.0-4.0 = very large and >4.0 = extremely large effects. Lacking information on the smallest meaningful effect for changes in protein phosphorylation and gene expression, the threshold for the smallest worthwhile effect

was defined as an ES of 0.4, rather than the conventional threshold of 0.2. Magnitude-based inferences about effects were made by qualifying the effects with probabilities reflecting the uncertainty in the magnitude of the true effect (Batterham & Hopkins, 2005). Effects deemed at least 75% 'likely' to be substantial (according to the overlap between the uncertainty in the magnitude of the true effect and the smallest worthwhile change (Batterham & Hopkins, 2005)) were included for analysis. Exact *P* values were also determined for each comparison, derived from paired (for within-group comparisons) or unpaired (for between-group comparisons) *t*-tests, with a Bonferroni correction applied to correct for multiple comparisons (SPSS, Version 21, IBM Corporation, New York, NY). Physiological (blood lactate, blood glucose, heart rate) and psychological (rating of perceived exertion [RPE]) responses to exercise are reported as mean values ± SD, whereas protein phosphorylation and gene expression data are reported as mean between-condition percentage differences ±90 % CL.

5.4 Results[5] [6]

5.4.1 Physiological and psychological responses to the post-training exercise bout

5.4.1.1 Heart rate and rating of perceived exertion (RPE)

During the post-training, single-bout exercise trial, there was a higher average heart rate (mean difference range, 14 ±12 to 19 ±14 bpm; ES, 1.04 ±0.88 to 1.22 ±0.89; $P \leq 0.043$; Table 5.3) and rating of perceived exertion (RPE) (2 ±2 to 4 ±2 AU; ES, 1.51 ±0.86 to 2.15 ±0.87; $P \leq 0.06$) for HIT compared with MICT.

5.4.1.2 Venous blood lactate and glucose responses

During the post-training, single-bout exercise trial, venous blood lactate (Table 5.3) was higher for HIT compared with MICT at all time points both during cycling (mean difference range, 0.8 ±0.5 to 4.5 ±1.1 $mmol \cdot L^{-1}$; ES range, 1.46 ±0.87 to 3.65

[5] All raw data for this Chapter is available in Appendix K.
[6] Extended within- and between-group comparison data for this chapter are presented in Appendices O and P, respectively.

±0.85; $P \leq 0.01$) and during the 15-min recovery period after cycling (3.5 ±1.0 to 5.0 ±1.2 mmol·L^{-1}; ES, 3.11 ±0.85 to 3.68 ±0.85; $P < 0.001$). Venous blood glucose (Table 5.3) was also higher for HIT compared with MICT after 16, 22, 28 and 34 min cycling (0.4 ±0.7 to 1.6 ±0.9 mmol·L^{-1}; ES, 0.54 ±0.86 to 1.52 ±0.86; $P \leq 0.039$), and during the 15-min recovery period after cycling (0.9 ±0.7 to 1.8 ±1.0 mmol·L^{-1}; ES, 1.11 ±0.85 to 1.50 ±0.85; $P \leq 0.041$).

After completion of the single-bout of RE, venous blood lactate (Table 5.4) was higher for HIT+RT compared with RT after 0, 2, 5, 10, 60, 90 and 180 min of recovery (0.1 ±0.1 to 1.4 ±0.9 mmol·L^{-1}; ES, 0.80 ±0.84 to 1.74 ±0.84; $P \leq 0.095$), and higher for HIT+RT compared with MICT+RT at all timepoints (0.1 ±0.1 to 1.1 ±1.4 mmol·L^{-1}; ES, 0.73 ±0.87 to 1.82 ±0.86; $P \leq 0.161$). Post-RE venous blood glucose (Table 5.4) was lower for HIT+RT compared with RT after 2, 10, and 30 min of recovery (0.3 ±0.2 to 0.3 ±0.3 mmol·L^{-1}; ES, -0.65 ±0.84 to -1.02 ±0.84; $P \leq 0.193$), and higher for HIT+RT compared with RT after 60 min of recovery (0.4 ±0.4 mmol·L^{-1}; ES, 0.88 ±0.84; $P = 0.077$). Blood glucose was higher for MICT compared with HIT+RT at +30 min of recovery (0.3 ±0.2 mmol·L^{-1}; ES, 1.29 ±0.86; $P = 0.021$), and lower for HIT+RT compared with MICT+RT at +60 min of recovery (0.2 ±0.2 mmol·L^{-1}; ES, -1.09 ±0.85; $P = 0.045$).

Table 5.3 Physiological and psychological (RPE) responses to a single bout of high-intensity interval training (HIT) or work-matched moderate-intensity continuous training (MICT) performed during the Study 2 post-training, single-bout exercise trial.

	Time (min)									
	Rest	10	16	22	28	34	+2	+5	+10	+15
Lactate ($mmol \cdot L^{-1}$)										
HIT	0.7 ± 0.3	2.6 ± 0.6 *#	5.4 ± 1.4 *#	6.8 ± 1.2 *#	7.3 ± 1.4 *#	7.3 ± 1.3 *#	7.3 ± 1.8 *#	7.2 ± 1.6 *#	6.0 ± 1.5 *#	4.9 ± 1.4 *#
MICT	0.7 ± 0.3	1.7 ± 0.5 *	2.6 ± 0.8 *	2.7 ± 0.8 *	2.8 ± 0.9 *	2.8 ± 1.0 *	2.4 ± 0.8 *	2.2 ± 0.8 *	1.8 ± 0.7 *	1.4 ± 0.5 *
Glucose ($mmol \cdot L^{-1}$)										
HIT	4.7 ± 0.8	4.6 ± 0.9	4.8 ± 0.9	5.0 ± 0.9 #	5.4 ± 1.1 #	5.9 ± 1.2 *#	6.3 ± 1.5 *#	6.2 ± 1.3 *#	5.9 ± 1.2 *#	5.4 ± 1.0 #
MICT	4.5 ± 0.5	4.5 ± 0.4	4.4 ± 0.6	4.2 ± 0.3	4.3 ± 0.4	4.3 ± 0.4	4.5 ± 0.5	4.7 ± 0.4	4.6 ± 0.4	4.5 ± 0.4
Heart rate ($beats \cdot min^{-1}$)										
HIT	63 ± 11	154 ± 9 *#	162 ± 9 *#	166 ± 9 *#	170 ± 10 *#	173 ± 9 *#	-	-	-	-
MICT	66 ± 5	140 ± 6 *	147 ± 17 *	150 ± 16 *	152 ± 17 *	154 ± 17 *	-	-	-	-
RPE (AU)										
HIT	6 ± 0	13 ± 3 *	15 ± 3 *#	17 ± 2 *#	18 ± 2 *#	18 ± 2 *#	-	-	-	-
MICT	6 ± 0	11 ± 2 *	12 ± 2 *	13 ± 2 *	14 ± 2 *	14 ± 2 *	-	-	-	-

Values are means ± SD. HIT, = high-intensity interval training cycling; MICT, continuous cycling; RPE, rating of perceived exertion. *, $P < 0.05$ vs. rest; #, $P < 0.05$ vs. MICT at same time point.

Table 5.4 Venous blood lactate and glucose responses to a single bout of resistance exercise (RE) either performed alone (RT) or when performed after either high-intensity interval training (HIT+RT) or work-matched moderate-intensity continuous training (MICT+RT) during the Study 2 post-training, single-bout exercise trial.

	Time (min)							
	End	+2	+5	+10	+30	+60	+90	+180
Lactate ($mmol \cdot L^{-1}$)								
RT	2.1 ± 0.7 *	2.3 ± 0.9 *	2.2 ± 1.0 *	1.7 ± 0.8 *	1.3 ± 1.3	0.7 ± 0.3	0.6 ± 0.2	0.5 ± 0.2
HIT+RT	3.5 ± 1.3 *‡	3.6 ± 1.5 *	3.3 ± 1.4 *	2.6 ± 1.2 *	1.6 ± 0.4 *#	1.2 ± 0.3 *#‡	0.8 ± 0.1 #‡	0.7 ± 0.1
MICT+RT	2.4 ± 1.2 *	2.5 ± 1.4 *	2.2 ±1.2 *	1.7 ± 0.7 *	0.9 ± 1.3	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.2
Glucose ($mmol \cdot L^{-1}$)								
RT	4.7 ± 0.3	4.7 ± 0.4	4.7 ± 0.4	4.7 ± 0.4	4.7 ± 0.3 ^	4.3 ± 0.5	4.5 ± 0.3	4.5 ± 0.2
HIT+RT	4.5 ± 0.9	4.5 ± 0.4	4.5 ± 0.4	4.4 ± 0.4	4.5 ± 0.2	4.7 ± 0.3 #	4.5 ± 0.2	4.6 ± 0.3
MICT+RT	4.6 ± 0.3	4.6 ± 0.3	4.7 ± 0.2	4.6 ± 0.2	4.7 ± 0.2 ^	4.4 ± 0.1	4.4 ± 0.2	4.4 ± 0.4

Values are means ± SD. HIT+RT, = high-intensity interval training cycling and resistance training; MICT+RT, continuous cycling and resistance training; RT, resistance training; *, $P < 0.05$ vs. rest; #, $P < 0.05$ vs. MICT at same time point; ^, $P < 0.05$ vs. HIT at same time point.; ‡, $P < 0.05$ vs. RT at same time point.

5.4.2 Signalling responses to training and the post-training exercise bout

5.4.2.1 Ribosome biogenesis signalling

p-TIF-1A^{Ser649}. There was a main effect of time for TIF-1A^{Ser649} phosphorylation ($P < 0.001$). At POST, TIF-1A phosphorylation was higher compared with PRE for HIT+RT (133 ±102%; ES, 0.62 ±0.31; $P = 0.047$; Figure 5.2A), but unchanged for RT or MICT+RT. Compared with POST, TIF-1A phosphorylation was higher for RT at +1 h (123 ±79%; ES, 0.45 ±0.19; $P = 0.002$), and +3 h (241 ±315%; ES, 0.69 ±0.46; $P = 0.017$), but unchanged for HIT+RT or MICT+RT. The change in TIF-1A phosphorylation between POST and +3 h was greater for RT compared with both HIT+RT (52 ±46%; ES, 0.76 ±0.89) and MICT+RT (75 ±24%; ES, 1.31 ±0.80), and lower for HIT+RT vs. MICT+RT (-47 ±36%; ES, -0.69 ±0.70).

TIF-1A protein. There was a main effect of time for changes in TIF-1A protein content ($P = 0.002$). Protein content of TIF-1A was reduced for HIT+RT at POST compared with PRE (-16 ±12%; ES, -0.17 ±0.14; $P = 0.047$; Figure 5.2B); however, the magnitude of this effect was very unlikely to be substantial. TIF-1A protein content was also reduced for RT at +3 h compared with POST (-42 ±19%; ES, 0.70 ±0.42; $P = 0.010$), and there was a likely larger reduction in TIF-1A protein content between POST and +1 h for HIT+RT vs. MICT+RT (-52 ±60%; ES, -1.65 ±2.38).

p-UBF^{Ser388}. There were main effects of time ($P < 0.001$), group ($P = 0.004$), and a time × group interaction ($P < 0.001$) for changes in UBF^{Ser388} phosphorylation. The phosphorylation of UBF^{Ser388} was unchanged at POST compared with PRE for all training groups (see Figure 5.2C). Compared with POST, UBF phosphorylation was increased for RT at both +1 h (78 ±58%; ES, 0.82 ±0.45; $P = 0.010$) and + 3 h (125 ±72%; ES, 1.15 ±0.45; $P = 0.001$), but unchanged for either HIT+RT or MICT+RT. The change in UBF phosphorylation between POST and +1 h was greater for RT compared with both HIT+RT (32 ±23%; ES, 0.54 ±0.46) and MICT+RT (37 ±27%; ES, 0.61 ±0.55), and greater between POST and +3 h for RT compared with both HIT+RT (49 ±17%; ES, 0.92 ±0.45) and MICT+RT (64 ±12%; ES, 1.35 ±0.42).

UBF protein. Protein content of UBF was increased for MICT+RT at POST compared with PRE (18 ±14%; ES, 0.44 ±0.31; P = 0.023; Figure 5.2D), but was not substantially changed for either HIT+RT or RT.

Cyclin D1 protein. There were main effects of time (P < 0.001) and group (P = 0.008) for changes in cyclin D1 protein content. Protein content of cyclin D1 was unchanged between PRE and POST for all training groups (Figure 5.2E). For HIT+RT, cyclin D1 protein content was reduced at +1 h compared with POST (-34 ±7%; ES, -0.66 ±0.16; P = 0.008).

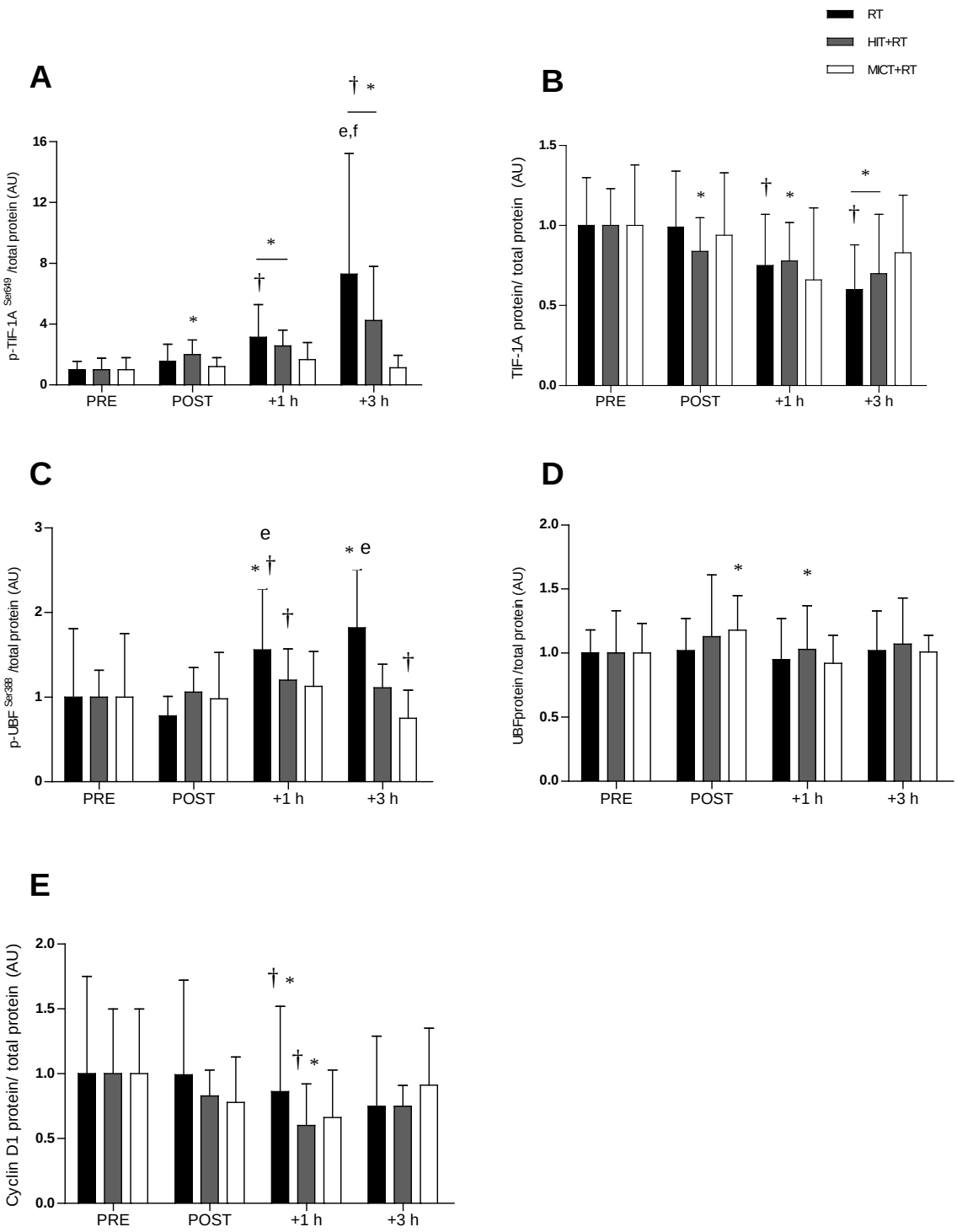

Figure 5.2 Phosphorylation of TIF-1A^{Ser649} (3A), UBF^{Ser388} (3C), and total protein content of TIF-1A (3B), UBF (3D), and cyclin D1 (3E) before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for all groups. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. POST. Change from POST substantially greater vs. e = HIT+RT, f = MICT+RT.

5.4.2.2 AMPK/mTORC1 signalling

p-AMPK^{Thr172}. There was a main effect of time for AMPK^{Thr172} phosphorylation (P = 0.033). The phosphorylation of AMPK^{Thr172} was unchanged at POST compared with PRE for all training groups (Figure 5.3A). AMPK phosphorylation was, however, increased at +1 h compared with POST for RT (78 ±72%; ES, 0.34 ±0.23; P = 0.031). The change in AMPK phosphorylation between POST and +3 h was also greater for RT compared with MICT+RT (59 ±44%; ES, 0.79 ±0.83) but not HIT+RT (54 ±49%; ES, 0.69 ±0.83).

AMPK protein. There were main effects of time (P = 0.008) and group (P = 0.001) changes in AMPK protein content. Protein content of AMPK was increased for HIT+RT at POST compared with PRE (32± 16%; ES, 0.63 ±0.28; P = 0.091), but was not substantially changed for either MICT+RT or RT.

p-ACC^{Ser79}. There was a time × group interaction for ACC^{Ser79} phosphorylation (P = 0.04).The phosphorylation of ACC^{Ser79} was unchanged at POST compared with PRE for all training groups (Figure 5.3B). Compared with POST, ACC phosphorylation was reduced at +1 h for both RT (-36 ±22%; ES, -0.28 ±0.20; P = 0.026) and MICT+RT (46 ±20%; ES, -0.56 ±0.33; P = 0.016), and reduced at +3 h compared with POST for RT (45 ±20%; ES, -0.37 ±0.22; P = 0.012). Compared with RT, the change in ACC phosphorylation was also greater for HIT+RT between POST and both +1 h (99 ±100%; ES, 0.65 ±0.46) and +3 h (169 ±168%; ES, 0.94 ±0.56).

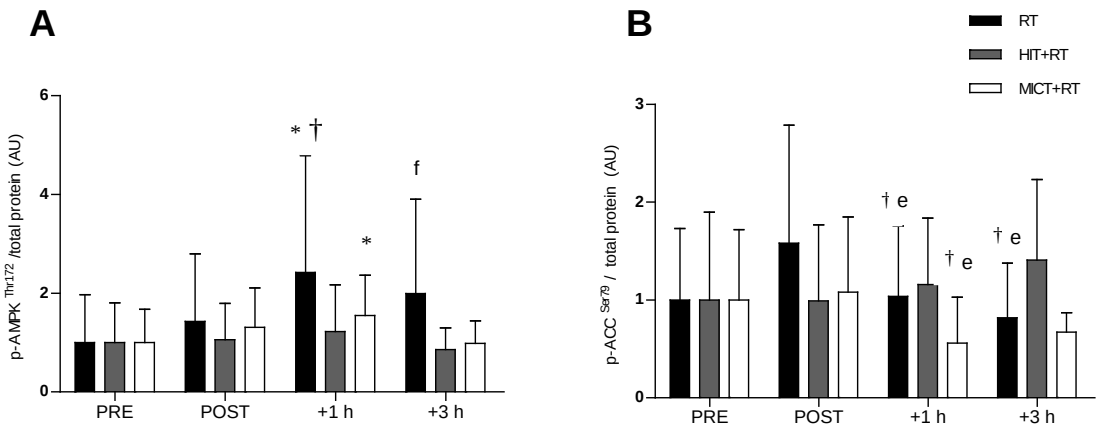

Figure 5.3 Phosphorylation of AMPK^{Thr172} (A) and ACC^{Ser79} (B) before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for all groups. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. POST. Change from POST substantially greater vs. e = HIT+RT, f = MICT+RT.

p-mTOR^{Ser2448}. There was a main effect of time for mTOR ^{Ser2448} phosphorylation (*P* = 0.001). The phosphorylation of mTOR^{Ser2448} was unchanged at POST compared with PRE for all training groups (Figure 5.4A). Compared with POST, mTOR phosphorylation was increased at +1 h for RT (105 ±137%; ES, 0.46 ±0.40; *P* = 0.048), but not for either HIT+RT (30 ±71%; ES, 0.32 ±0.62; *P* = 0.320) or MICT+RT (77 ±184%; ES, 0.37 ±0.59; *P* = 0.218), and increased at +3 h for compared with POST for HIT+RT (70 ±45%; ES, 0.64 ±0.31; *P* = 0.030). There were no substantial between-group differences in mTOR phosphorylation at any time point.

p-p70S6K1^{Thr389}. There was a main effect of time for p70S6K1^{Thr389} phosphorylation (*P* < 0.001). The phosphorylation of p70S6K1^{Thr389} was increased at POST compared with PRE for HIT+RT (95 ±47%; ES, 0.66 ±0.24; *P* = 0.024; Figure 5.4B). Compared with POST, p70S6K1 phosphorylation was increased by RT at +1 h (78 ±77%; ES, 0.51 ±0.37; *P* = 0.026) but was unchaned for HIT+RT or MICT+RT. The change in p70S6K1 phosphorylation between POST and +3 h was also substantially greater for RT compared with both HIT+RT (47 ±50%; ES, 0.86 ±1.13) and MICT+RT (50 ±46%; ES, 0.88 ±1.05).

p-rps6$^{Ser235/236}$. There was a main effect of time for rps6$^{Ser235/236}$ phosphorylation (*P* < 0.001). The phosphorylation of rps6$^{Ser235/236}$ was unchanged at

POST compared with PRE for all training groups (Figure 5.4C). Compared with POST, rps6 phosphorylation was increased for all training groups at +1 h (RT: 700 ±678%; ES, 0.75 ±0.28; $P < 0.001$; HIT+RT: 475 ±572%; ES, 0.66 ±0.33; $P = 0.005$; MICT+RT: 621 ±420%; ES, 1.49 ±0.42; $P < 0.001$) and +3 h (RT: 967 ±1047%; ES, 0.85 ±0.31; $P < 0.001$; HIT+RT: 294 ±319%; ES, 0.51 ±0.28; $P = 0.006$; MICT+RT: 176 ±200%; ES, 0.76 ±0.51; $P = 0.026$). The change in rps6 phosphorylation between POST and +3 h was, however, substantially greater for RT compared with MICT+RT (74 ±29%; ES, 0.72 ±0.51) but not HIT+RT (63 ±41%; ES, 0.57 ±0.56).

p-4E-BP1$^{Thr56/47}$. There was a main effect of group for 4E-BP1$^{Thr36/47}$ phosphorylation ($P < 0.001$). The phosphorylation of 4E-BP1$^{Thr36/47}$ was increased for MICT+RT at +3 h compared with PRE (59 ±54%; ES, 0.56 ±0.40; $P = 0.027$; Figure 5.4D), but unchanged for either HIT+RT or RT at any time point.

p-eEF2^{Thr56}. There was a main effect of time for eEF2^{Thr56} phosphorylation ($P = 0.004$). The phosphorylation of eEF2^{Thr56} was unchanged at POST compared with PRE for all training groups (Figure 5.4E). eEF2 phosphorylation was reduced at + 1 h compared with POST for HIT+RT (-43 ±11%; ES, -0.71 ±0.25; $P = 0.001$). There were no substantial between-group differences in changes in eEF2 phosphorylation.

p-GSK-3β^{Ser9}. There were main effects of time ($P = 0.007$) and group ($P < 0.001$) for GSK-3β^{Ser9} phosphorylation. The phosphorylation of GSK-3β^{Ser9} was increased at POST compared with PRE for MICT+RT (23 ±20%; ES, 0.37 ±0.29; $P = 0.034$; Figure 5.4F).

ACC, mTOR, p70S6K1, rps6, GSK-3β and 4E-BP1 protein. There were no substantial changes in the total protein content of either ACC, mTOR, p70S6K1, rps6, GSK-3β or 4E-BP1 at any time point (data not shown).

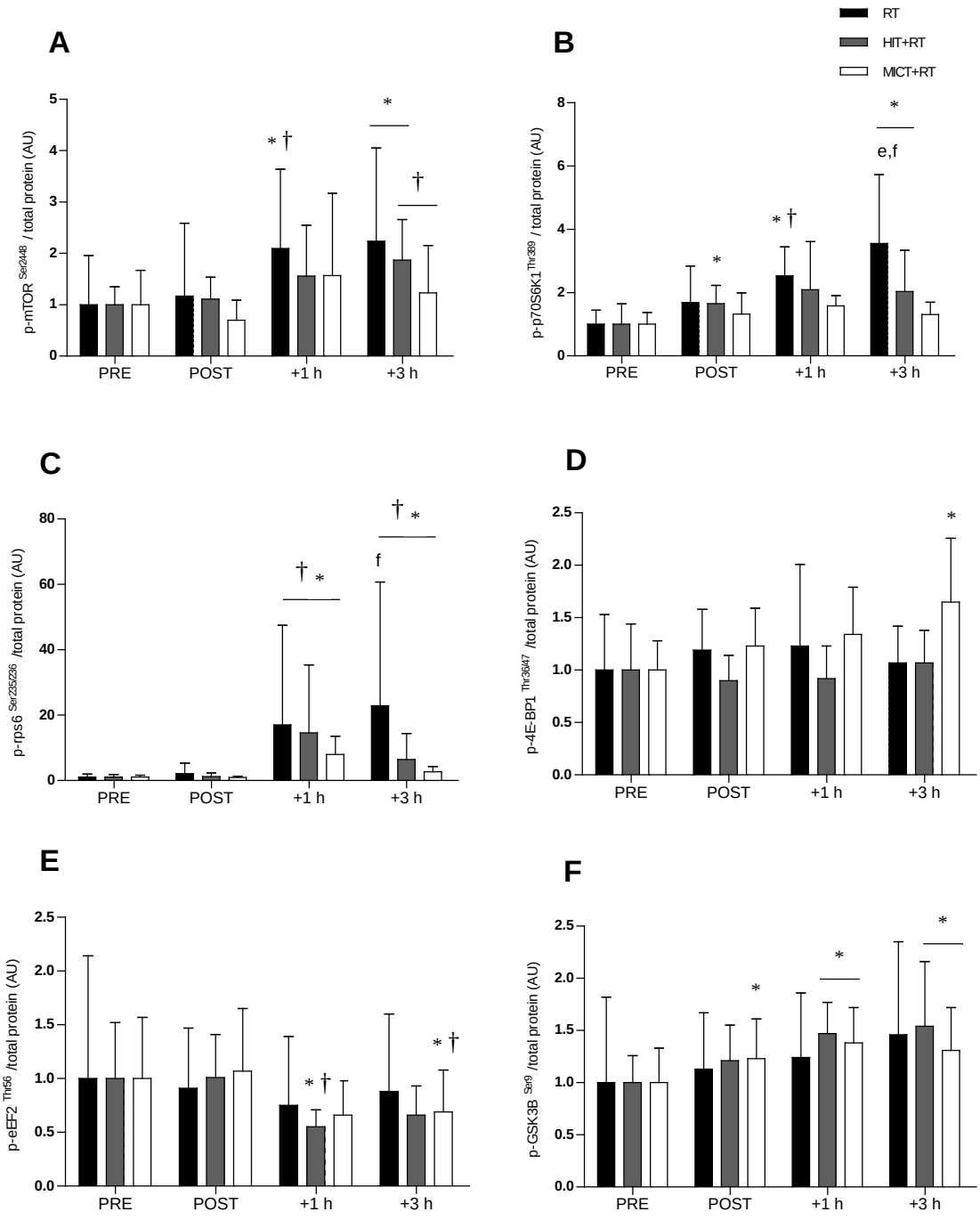

Figure 5.4 Phosphorylation of mTOR^{Ser2448} (A), p70S6K^{Thr389} (B), rps6$^{Ser235/236}$ (C), 4E-BP1$^{Thr36/47}$ (D), eEF2^{Thr56} (E), and GSK-3β^{Ser9} (F) before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for all groups. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. POST. Change from POST substantially greater vs. e = HIT+RT, f = MICT+RT

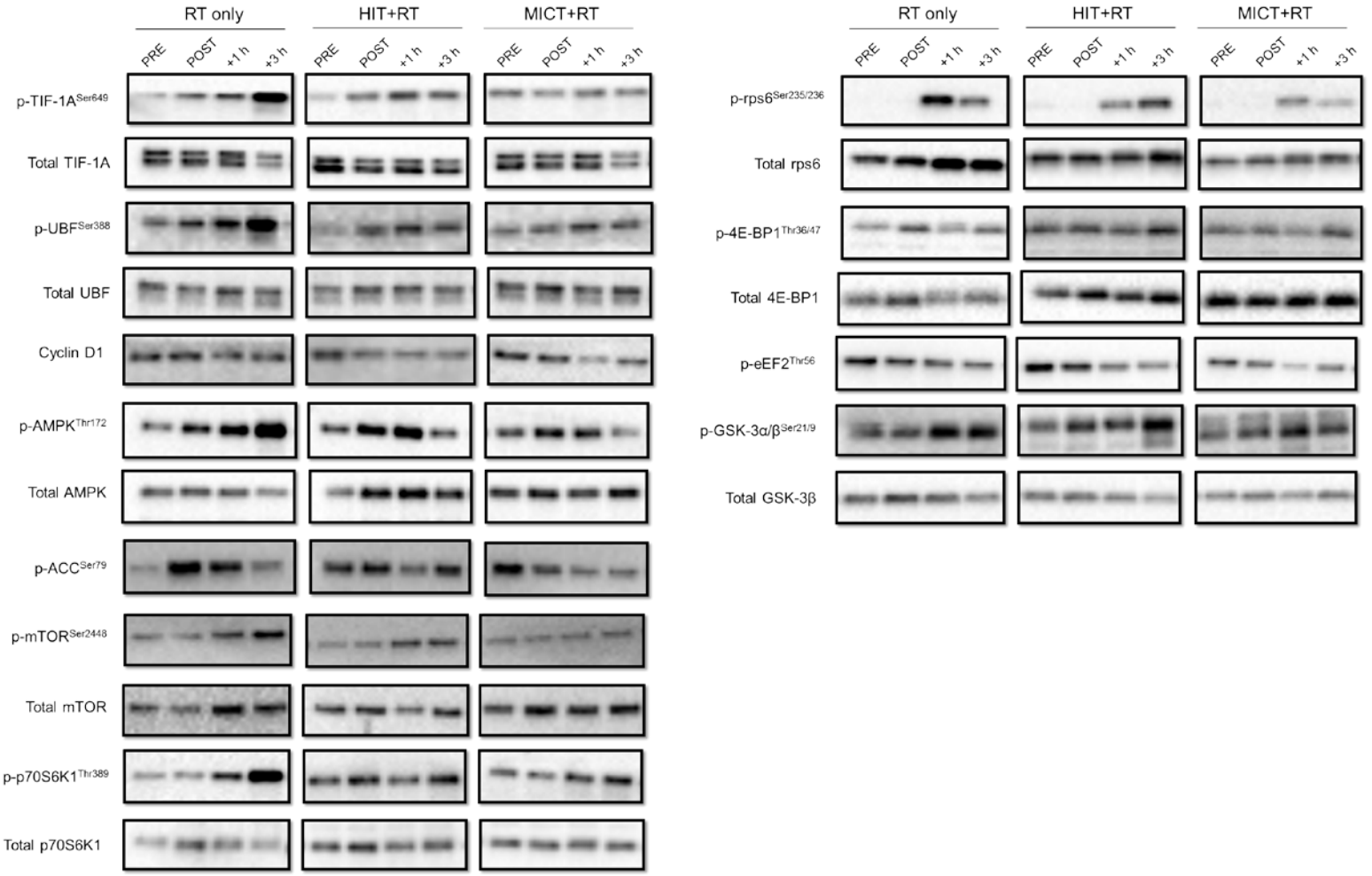

Figure 5.5 Representative western blots for the phosphorylation (p-) and total protein content of signalling proteins before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a training group-specific exercise bout performed after completion of the training intervention.

5.4.3 Ribosomal RNA (rRNA) responses to training and the post-training exercise bout

Total RNA content. Total RNA content was used as an index of total translational capacity of skeletal muscle, since ribosomal RNA comprises over 85% of the total RNA pool (Haddad et al., 2005). There was a time × group interaction for changes in total RNA content (P = 0.004). At PRE, total RNA content was higher for RT compared with both HIT+RT (81 ±76%; ES, -0.71 ±0.49; P = 0.026; Figure 5.6A) and MICT+RT (63 ±94%; ES, 0.59 ±0.66; P = 0.052). Total RNA content decreased between PRE and POST for RT (-25 ±11%; ES, -0.19 ±0.09; P = 0.030); however, the magnitude of this effect was most unlikely to be substantial. Conversely, total RNA content was increased between PRE and POST for HIT+RT (47 ±15%; ES, 0.39 ±0.10; P = 0.023) but was not substantially different for MICT+RT (27 ±26%; ES, 0.08 ±0.07; P = 0.060). The PRE-POST change in total RNA content was greater for both HIT+RT (106 ±67%; ES, 1.35 ±0.60) and MICT+RT (69 ±45%; ES, 1.05 ±0.53) compared with RT.

The training induced change in total RNA content was negatively correlated with baseline total RNA content (r = -0.885, P < 0.001; Figure 5.6B). Total RNA content at POST was also negatively correlated with the training-induced change in lower body lean mass measured via DXA (as reported in Table 4.3; r = -0.600, P = 0.002).

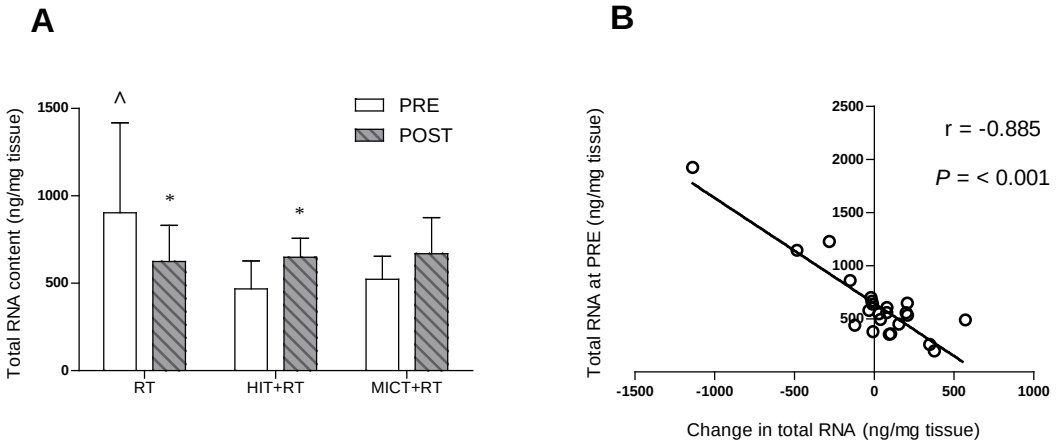

Figure 5.6 Total RNA content (A) of the vastus lateralis before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and the correlation between total RNA content at PRE and the training-induced change in total RNA content (B). Data presented are means ± SD. * = P < 0.05 vs. PRE, ^ = P < 0.05 vs. both HIT+RT and MICT+RT at PRE.

45S pre-rRNA. There was a main effect of time for changes in 45S pre-rRNA expression (P < 0.001). Expression of 45S pre-rRNA was unchanged at POST compared with PRE for all training groups (Figure 5.7); however, the change in 45S pre-rRNA expression between PRE and POST was greater for both HIT+RT (58 ±76%; ES, 0.71 ±0.71) and MICT+RT (75 ±81%; ES, 0.85 ±0.68) compared with RT. There were no substantial changes nor between-group differences in 45S pre-rRNA expression between POST and +3 h for either training group.

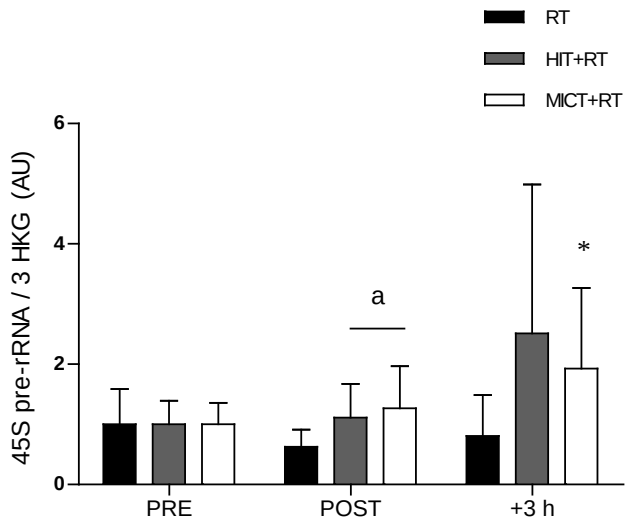

Figure 5.7 Expression of 45S pre-rRNA relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for all groups. * = P < 0.05 vs. PRE, a = change between PRE and POST substantially greater vs RT.

5.8S rRNA (mature). There was a main effect of time for changes in 5.85S rRNA expression (P = 0.004). Expression of 5.85S rRNA was reduced at POST compared with PRE for RT (-51 ±16%; ES, -0.69 ±0.31; P = 0.017; Figure 5.8A). The change in 5.8S rRNA expression between PRE and POST was also greater for both HIT+RT (125 ±109%; ES, 1.27 ±0.73) and MICT+RT (120 ±111%; ES, 0.99 ±0.61) compared with RT. There were no substantial changes in 5.8S rRNA expression between POST and +3 h for either training group.

5.8S rRNA (span). There was a time × group interaction for changes in 5.85S (span) rRNA expression (P = 0.008). Expression of 5.8S rRNA (span) was reduced at POST compared with PRE for RT (-36 ±15%; ES, -0.51 ±0.27; P = 0.027; Figure 5.8B). The change in 5.8S rRNA (span) expression between PRE and POST was also greater for HIT+RT compared with RT (112 ±116%; ES, 1.40 ±0.97).

18S rRNA (mature). There was a main effect of group for changes in 5.85S rRNA expression (P = 0.049). Expression of 18S rRNA was, however, not substantially different at any time point, nor were there any substantial between-group differences in changes in 18S rRNA expression (Figure 5.8C).

18S rRNA (span). There were no substantial effects of training or any between-group differences in changes in 18S rRNA (span) expression (Figure 5.8D), although a small increase in 18S rRNA (span) expression was noted at +3 h compared with POST for MICT+RT (63 ±48%; ES, 0.21 ±0.12; P = 0.029).

28S rRNA (mature). Expression of 28S rRNA was reduced at POST compared with PRE for RT (-33 ±15%; ES, -0.49 ±0.28; P = 0.037; Figure 5.8E); however, this effect was only possibly substantial. The change in 28S rRNA expression between PRE and POST was also greater for both HIT+RT (73 ±56%; ES, 1.23 ±0.71; P = 0.007) and MICT+RT (63 ±55%; ES, 1.10 ±0.74; P = 0.023) compared with RT. There were no substantial changes in 28S rRNA expression between POST and +3 h for either training group.

28S rRNA (span). There was a main effect of group for changes in 28S rRNA (span) expression (P < 0.001). There were no substantial changes in 28S rRNA (span) expression between PRE and POST for either training group (Figure 5.8F). However, the change in 28S rRNA (span) expression between PRE and POST was greater for HIT+RT compared with RT (123 ±109%; ES, 0.81 ±0.48).

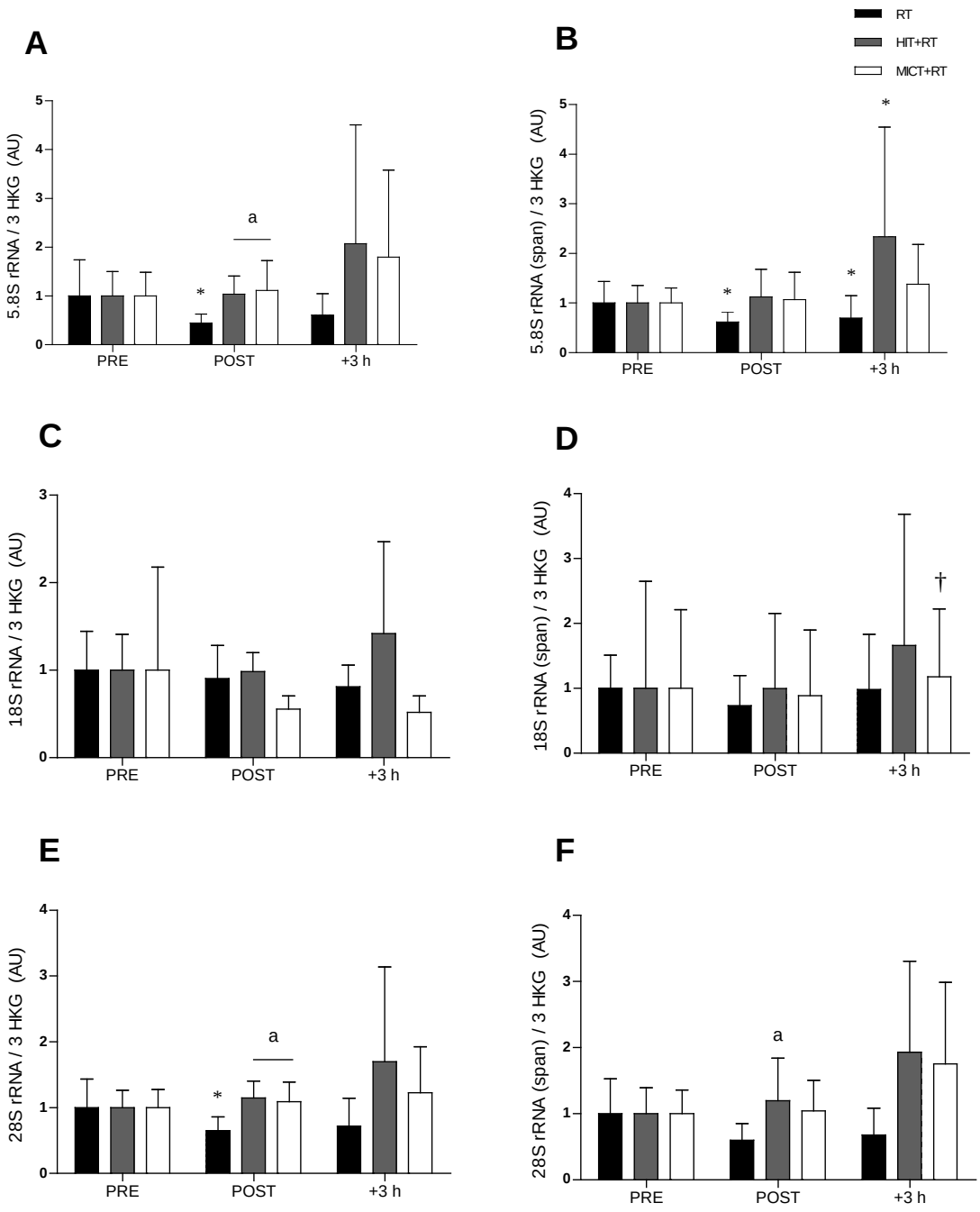

Figure 5.8 Expression of the mature rRNA transcripts 5.8S rRNA (A), 18S rRNA (C), and 28S rRNA (E), and rRNA transcripts bound to the 45S pre-RNA precursor: 5.8S rRNA (span) (B) 18S rRNA (span) (D) and 28S rRNA (span) (F) relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for all groups. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. POST, a = change between PRE and POST substantially greater vs RT.

5.4.4 mRNA responses to training and the post-training exercise bout

TIF-1A mRNA. There was a main effect of time for changes in TIF-1A mRNA expression (P = 0.008). Expression of TIF-1A mRNA was unchanged at POST compared with PRE for all training groups (Figure 5.9A). Compared with POST, TIF-1A expression was increased at +3 h for both RT (26 ±12%; ES, 0.53 ±0.21; P = 0.003) and MICT+RT (36 ±35%; ES, 0.59 ±0.50; P = 0.038), but not HIT+RT. There were no substantial between-group differences in changes in TIF-1A expression.

UBF mRNA. There were main effects of time (P = 0.008) and group (P = 0.039) for changes in UBF mRNA expression. Expression of UBF mRNA was unchanged at POST compared with PRE for all training groups (Figure 5.9B). There were no substantial changes in UBF expression between POST and +3 h for either training group.

POLR1B mRNA. There were main effects of time (P = 0.001) and a time × group interaction (P = 0.007) for changes in POLR1B mRNA expression. Expression of POLR1B mRNA was reduced at POST compared with PRE for RT (-26 ±16%; ES, -0.44 ±0.32; P = 0.026; Figure 5.9C); however, this effect was only 'possibly' substantial. Compared with POST, POLR1B expression was increased at +3 h for both HIT+RT (44 ±42%; ES, 0.57 ±0.44; P = 0.047) and MICT+RT (48 ±43%; ES, 0.51 ±0.37; P = 0.033), but unchanged for RT. The change in POLR1B mRNA expression between both PRE-POST (37 ±30%; ES, 0.87 ±0.60) and POST-+3 h (34 ±51%; ES, 0.81 ±1.03) was greater for HIT+RT vs. RT.

Cyclin D1 mRNA. There was a main effect of time for changes in cyclin D1 mRNA expression (P = 0.007). Expression of cyclin D1 mRNA was increased for HIT+RT at POST compared with PRE (101 ±54%; ES, 0.59 ±0.22; P = 0.001; Figure 5.9D). There were no substantial changes in cyclin D1 mRNA expression between POST and +3 h for either training group.

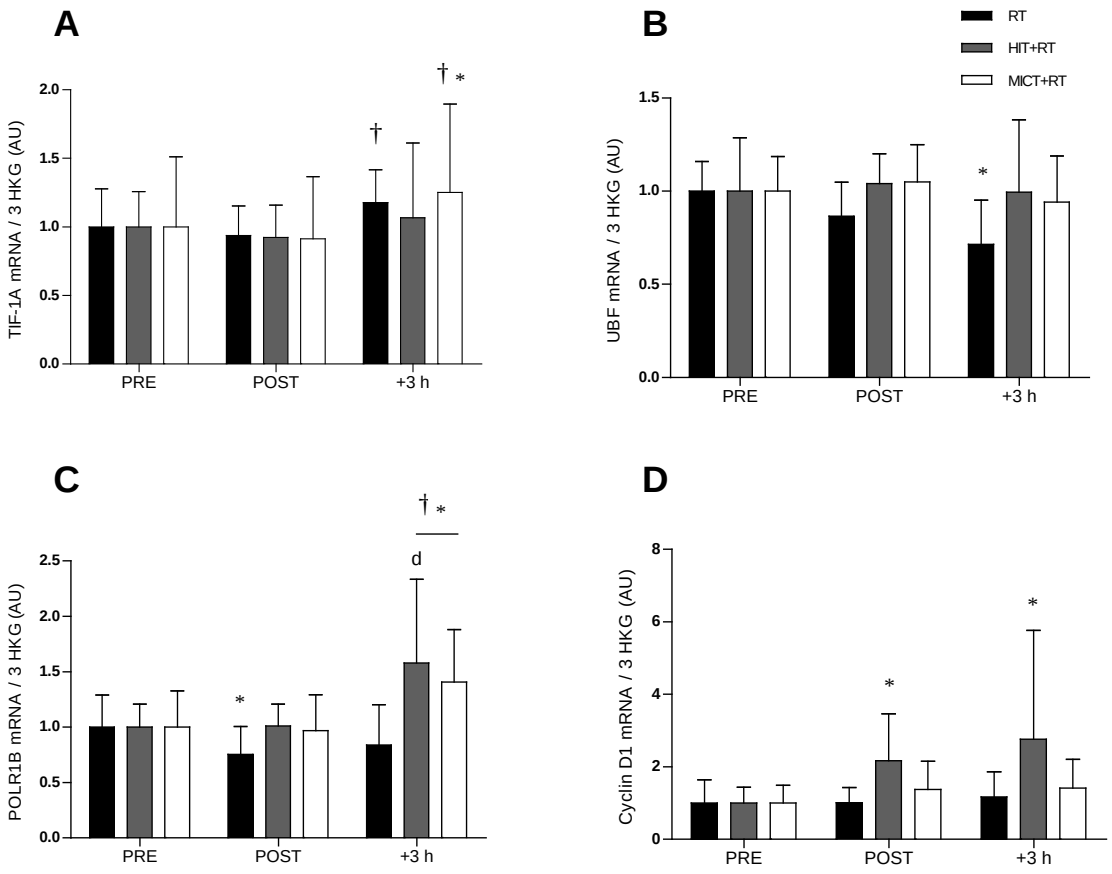

Figure 5.9 mRNA expression of TIF-1A (A), UBF (B), POLR1B (C), and cyclin D1 (D) relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for all groups. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. POST. Change from POST substantially greater vs. d = RT.

MuRF-1 mRNA. There were main effects of time (P = 0.004) and a time × group interaction (P = 0.019) for changes in MuRF-1 mRNA expression. Expression of MuRF-1 mRNA was unchanged at POST compared with PRE for all training groups (Figure 5.10A). Compared with POST, MuRF-1 expression was increased at +3 h for HIT+RT (206 ±163%; ES, 1.35 ±0.61; P = 0.003), but unchanged for either MICT+RT and RT. The change in MuRF-1 expression between POST and +3 h was greater for HIT+RT compared with both RT (168 ±176%; ES, 2.15 ±1.34) and MICT+RT (60 ±34%; ES, 1.85 ±1.56).

Atrogin-1 mRNA. There were main effects of time (P = 0.028) and a time × group interaction (P = 0.049) for changes in Atrogin-1 mRNA expression. Expression of Atrogin-1 mRNA was unchanged at POST compared with PRE for all training groups (Figure 5.10B). Compared with POST, Atrogin-1 expression was reduced at +3 h for RT (-44 ±22%; ES, -0.91 ±0.60; P = 0.018), but not substantially changed for either HIT+RT ot MICT+RT. The reduction in Atrogin-1 mRNA expression between POST and +3 h was greater for RT compared with both HIT+RT (-89 ±83%; ES, -1.22 ±0.82) and MICT+RT (-86 ±89%; ES, -1.14 ±0.85).

Fox-O1 mRNA. There was a main effect of time for changes in Fox-O1 mRNA expression (P = 0.004).The mRNA levels of Fox-O1 were unchanged between PRE and POST for all training groups (Figure 5.10C). At +3 h, Fox-O1 mRNA was increased compared with POST only for HIT+RT (158 ±65%; ES, 0.59 ±0.16; P < 0.001). The change in Fox-O1 mRNA expression between POST and +3 h was also substantially greater for HIT+RT compared with both RT (141 ±73%; ES, 0.80 ±0.27) and MICT+RT (47 ±31%; ES, 0.54 ±0.47).

PGC-1α mRNA. There were main effects of time (P < 0.001), group (P < 0.001), and a time × group interaction (P < 0.001) for changes in PGC-1α mRNA expression. Expression of PGC-1α mRNA was reduced between PRE and POST for RT (-26 ±14%; ES, -0.48 ±0.30; P = 0.026; Figure 5.10D) and MICT+RT (-45 ±13%; ES, -0.61 ±0.23; P = 0.157), but unchanged at POST for HIT+RT. Compared with POST, PGC-1α mRNA expression was increased at +3 h for both HIT+RT (826 ±349%; ES, 4.58 ±0.76; P < 0.001) and MICT+RT (590 ±481%; ES, 1.97 ±0.66; P = 0.001), but unchanged for RT. The change in PGC-1α mRNA expression between POST and +3 h

was also greater for both HIT+RT (635 ±360%; ES, 4.80 ±1.14) and MICT+RT (447 ±379%; ES, 2.75 ±1.05) compared with RT.

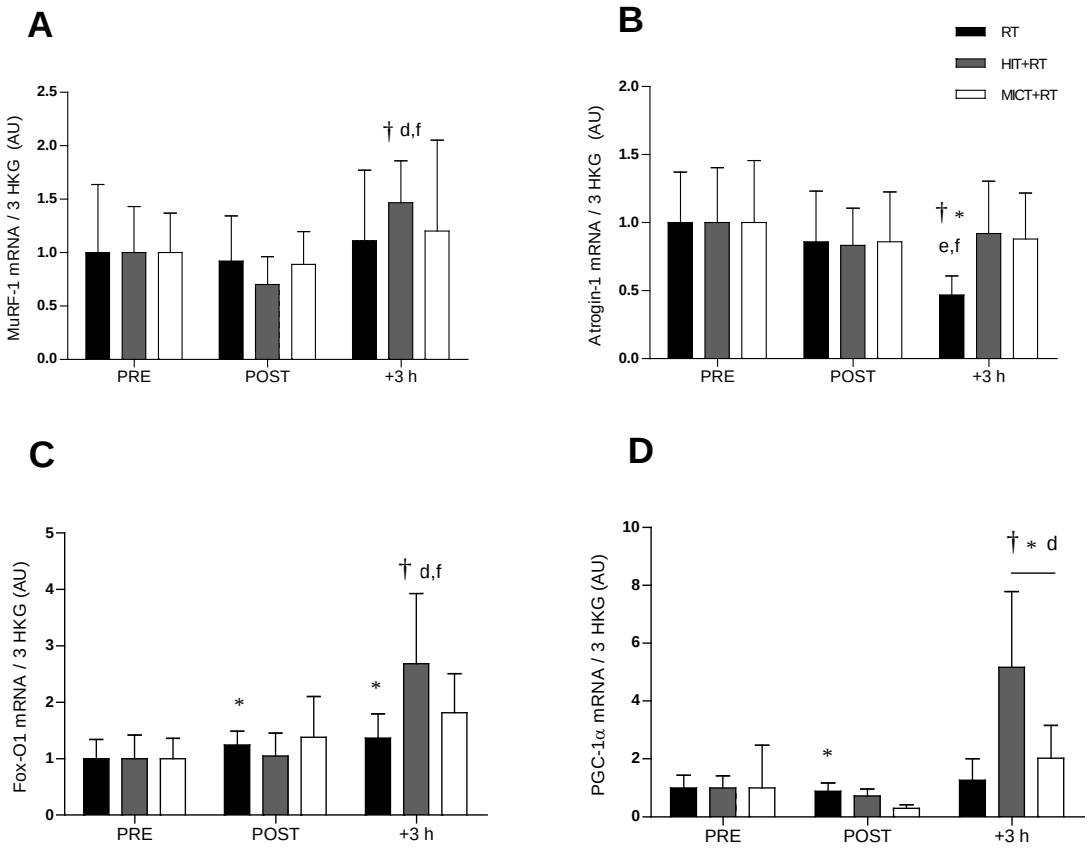

Figure 5.10 mRNA expression of MuRF-1 (A), Atrogin-1 (B), Fox-O1 (C) and PGC-1α (D) relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for all groups. * = *P* < 0.05 vs. PRE, † = *P* < 0.05 vs. POST. Change from POST substantially greater vs. d = RT, e = HIT+RT, f = MICT+RT.

5.4.5 Muscle fibre CSA responses to training

Type I muscle fibre CSA (Figure 5.11A) was increased at POST compared with PRE for RT (15 ±13%; ES, 0.10 ±0.08; *P* = 0.035), but was not substantially changed for either HIT+RT (-23 ±19%; ES, -0.09 ±0.08; *P* = 0.135) or MICT+RT (0.4 ±17%; ES, 0.00 ±-0.14; *P* = 0.989). The training-induced change in type I fibre CSA

was also substantially greater for RT compared with HIT+RT (34 ±22%; ES, 1.03 ±0.80), but not MICT+RT (15 ±54%; ES, 0.39 ±1.45).

Type II muscle fibre CSA (Figure 5.11B) was not substantially changed between PRE and POST for either RT (19 ±27%; ES, 0.09 ±0.12; P = 0.139), HIT+RT (0.4 ±24%; ES, 0.00 ±0.08; P = 0.974) or MICT+RT (16 ±14%; ES, 0.19 ±0.16; P = 0.344). There were no substantial differences in the training-induced changes in type II fibre CSA.

At POST, type I fibre CSA (r = 0.591; P = 0.005), type II fibre CSA (r = 0.470; P = 0.032), and combined type I and type II fibre CSA (r = 0.551; P = 0.010) were correlated with total skeletal muscle RNA content. Correlations were also noted between total RNA content at POST and the training-induced change in both type I fibre CSA (r = 0.454; P = 0.044) and combined type I and type II fibre CSA (r = 0.470; P = 0.032).

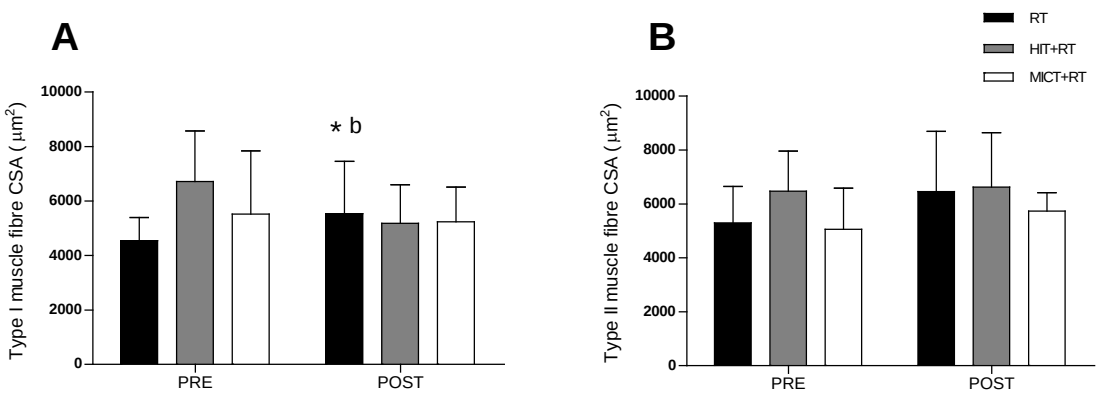

Figure 5.11 Changes in type I (A) and type II (B) muscle fibre CSA before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT). Data presented are means ± SD and expressed relative to the PRE value for all groups. * = P < 0.05 vs. PRE, b = change between PRE and POST substantially greater vs. HIT+RT.

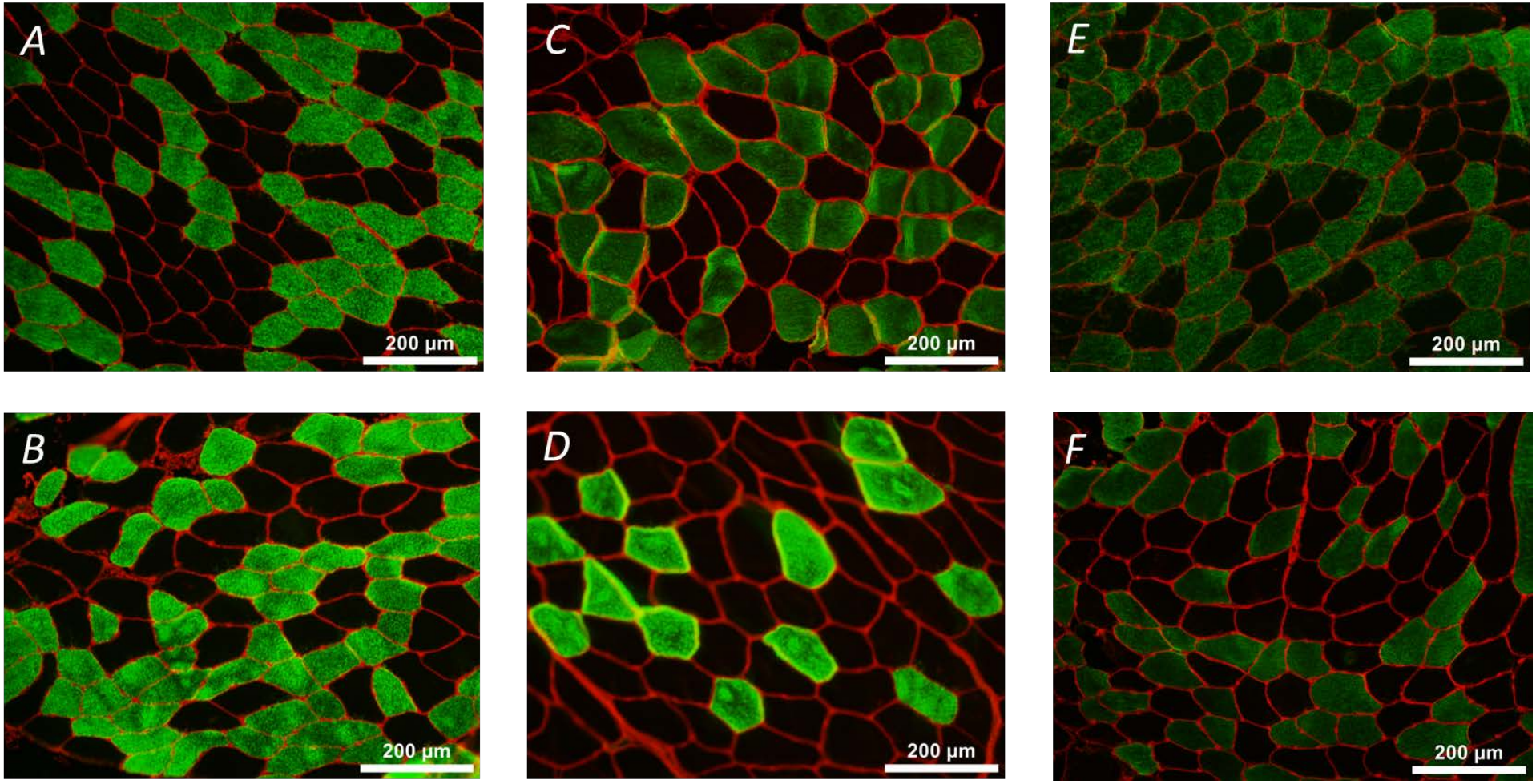

Figure 5.12 Representative immunohistochemical images of muscle cross-sections obtained before (PRE) and after (POST) eight weeks of either RT alone (images A and B, respectively), or RT combined with either high-intensity interval training (HIT+RT; images C and D, respectively) or moderate-intensity continuous training (MICT+RT; images E and F, respectively). Muscle fibre membranes are stained red, type I muscle fibres are stained green, and type II muscle fibres are unstained.

5.5 Discussion

Previous investigations on molecular responses and adaptations in skeletal muscle to concurrent training have focused almost exclusively on measures of increased translational efficiency (i.e., mTORC1 signalling and rates of MPS) (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Coffey et al., 2009a; Coffey et al., 2009b; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015). Novel data is presented on the regulation of translational capacity (i.e., ribosome biogenesis) with concurrent training relative to RT performed alone, including regulators of RNA Pol-I-mediated rDNA transcription, and changes in expression levels of the 45S rRNA precursor and mature rRNA species (5.8S, 18S, and 28S). The major findings were that although a single bout of RE performed in a training-accustomed state increased mTORC1 signalling and the phosphorylation of RNA Pol-I regulatory factors (TIF-1A and UBF) compared with concurrent training, this was not associated with increased expression of either the 45S rRNA precursor or mature rRNA species. Rather, changes in total RNA content and expression of mature rRNAs tended to be greater following concurrent exercise, regardless of the endurance training intensity employed, suggesting enhanced ribosome biogenesis. These observations contrasted our findings regarding training-induced changes in muscle fibre-type specific hypertrophy, which was attenuated in type I muscle fibres for the HIT+RT group, suggesting a disconnect between training-induced changes in markers of ribosome biogenesis and skeletal muscle hypertrophy.

Previous work has demonstrated concurrent exercise either does not compromise early post-exercise mTORC1 signalling or rates of MPS (Apro et al., 2015; Apro et al., 2013; Carrithers et al., 2007; Donges et al., 2012; Pugh et al., 2015), or rather potentiates these responses (Lundberg et al., 2012), compared with RE performed alone. Most of these studies have, however, examined these responses in either untrained individuals or those who are relatively unaccustomed to the exercise protocol. Given short-term training increases the mode-specificity of post-exercise molecular responses (Vissing et al., 2011; Wilkinson et al., 2008); examining perturbations to molecular signalling and gene expression in relatively training-unaccustomed individuals may confound any insight into interference to these responses following concurrent training (Fyfe et al., 2014). A post-training exercise trial was employed to investigate potential interference to mTORC1 signalling following exercise protocols

participants were accustomed to from eight weeks of prior training, thereby overcoming some of the limitations of most single-bout concurrent exercise studies (Carrithers et al., 2007; Donges et al., 2012; Lundberg et al., 2012; Pugh et al., 2015). In contrast to previous investigations (Carrithers et al., 2007; Donges et al., 2012; Lundberg et al., 2012; Pugh et al., 2015), evidence of enhanced mTORC1 signalling was observed after RT compared with concurrent exercise, including increased mTOR and p70S6K1 phosphorylation at 1 h post-exercise, and elevated rps6 phosphorylation at +3 h for RT when compared with both concurrent exercise trials. These observations contrast previous data (Fernandez-Gonzalo et al., 2013) showing no differences in mTORC1 signalling between single bouts of either RE, either performed alone or following a bout of continuous endurance exercise, both in trained and untrained states. The authors (Fernandez-Gonzalo et al., 2013) suggested, however, that any small tendency for mTORC1 signalling responses (e.g., p70S6K^{Thr389} phosphorylation) to be enhanced by concurrent exercise before training, as shown in a previous study (Lundberg et al., 2012), were lessened when exercise was performed in a training-accustomed state. Although post-exercise molecular responses to exercise were not measured in the present study before training, data presented in Chapter 3 demonstrated single bouts of concurrent exercise, performed in a relatively unfamiliar state, induce similar post-exercise mTORC1 signalling responses to RE performed alone. Together, these data lend support to the notion the molecular signals initiated in skeletal muscle by exercise become more mode-specific with repeated training, and early post-exercise mTORC1 signalling may be attenuated with concurrent exercise compared with RE, when performed in a training-accustomed state.

While the observed mTORC1 signalling responses were consistent with the paradigm of enhanced mode-specificity of molecular responses with repeated training, the finding of enhanced AMPK phosphorylation following RT compared with concurrent exercise was unexpected, given the energy-sensing nature of AMPK signalling and its purported role in promoting an oxidative skeletal muscle phenotype (McGee & Hargreaves, 2010). Potentially, this observation may suggest an adaptive response whereby endurance training rendered subjects in the concurrent training groups less susceptible to exercise-induced metabolic perturbation in skeletal muscle, manifesting in an attenuated post-exercise AMPK phosphorylation response. A similar phenomenon has been observed in human skeletal muscle after only 10 days of endurance training, whereby post-exercise increases in AMPK activity following a

single pre-training exercise bout are attenuated compared with the same exercise bout performed before training (McConell et al., 2005). The present data suggest further work is required to further define the mode-specificity of AMPK signalling in skeletal muscle and the effect of repeated training on induction of these responses.

In addition to mediating transient changes in translational efficiency, accumulating evidence suggests mTORC1 also plays a critical role in regulating ribosome biogenesis (and therefore translational capacity) in skeletal muscle by regulating all three classes of RNA polymerases (RNA Pol I-III) (Iadevaia et al., 2014). Inhibition of mTORC1 by rapamycin leads to the inactivation of TIF-1A, which impairs the recruitment of RNA Pol-I-associated transcription-initiation complexes mediating the transcription of 45S pre-rRNA genes (Mayer et al., 2004). The key mTORC1 substrate p70S6K1 also plays a role in mediating Pol-I activity via its interaction with UBF, a transcription factor that interacts with the RNA Pol-I machinery via SL-1 (Hannan et al., 2003). In agreement with mTORC1 signalling responses, the phosphorylation of upstream regulators of RNA Pol-I-mediated rDNA transcription, including UBF and TIF-1A, was further increased by RT alone than when combined with either HIT or MICT. Previous work (Figueiredo et al., 2015) has demonstrated single bouts of RT induces robust increases in TIF-1A Ser^{649} phosphorylation and UBF protein expression in human skeletal muscle at 1 h post-exercise, both in untrained and trained states. Moreover, whereas single bouts of RT did not impact upon UBF Ser^{388} phosphorylation, this response was elevated in the basal state post-training (Figueiredo et al., 2015). The present data add to the growing body of evidence that RT is a potent stimulus for increasing the phosphorylation of regulators of Pol-I-mediated rDNA transcription, and suggest these early signalling responses may be similarly attenuated when RT is combined with endurance exercise in the form of either HIT or MICT.

The regulation of several Pol-I associated proteins was also measured at the transcriptional level, including TIF-1A, POLR1B, UBF, and cyclin D1. The present data suggest concurrent exercise, irrespective of endurance training intensity, was a sufficient stimulus for increasing POLR1B mRNA expression at 3 h post-exercise, but only MICT+RT and RT alone increased TIF-IA mRNA content at this timepoint. Previous work in human skeletal muscle has demonstrated no effect of a single bout of RE performed in either untrained or trained states on the mRNA expression of either TIF-1A or POLR1B at either 1 h (Figueiredo et al., 2015) or 4 h (Nader et al., 2014) post-exercise. Eight weeks of RT has previously been shown to increase basal UBF

mRNA expression, which was reduced 1 h following a single RT bout post-training (Figueiredo et al., 2015). Although there were no basal training-induced increases in UBF mRNA expression for any training group in the present study, a similar reduction in UBF mRNA content was noted 3 h post-exercise for the RT group. Increased cyclin D1 mRNA was also seen at rest post-training for the HIT+RT group, which was maintained at 3 h post-exercise. Figueiredo et al. (2015) have shown eight weeks of RT decreased post-training levels of cyclin D1 mRNA compared with pre-training, with a small increase induced at 1 h post-exercise by a single bout of post-training RT. It therefore appears HIT is a more potent stimulus for increasing levels of cyclin D1 mRNA compared with RT alone or MICT, although an acute reduction in cyclin D1 protein levels was also seen 1 h following a single bout of HIT+RT. Previous work has shown increases in cyclin D1 mRNA during long-term (3 months) RT (Kadi et al., 2004), which may suggest an increase in satellite cell activation and proliferation during the training intervention (Adams et al., 1999; Kadi et al., 2004), although direct measures of these markers were not made in the present study.

Despite the present findings regarding signalling responses upstream of 45S pre-rRNA transcription, the expression of 45S pre-RNA, but not mature ribosome species, was acutely increased only by concurrent exercise and not RT alone. Previous work in humans has reported basal increases in 45S pre-rRNA after 8 weeks of RT (Figueiredo et al., 2015), and 4 h after a single bout of RT performed in both untrained and trained states (Nader et al., 2014). Notably, expression of 45S pre-rRNA was less pronounced in the trained state compared with untrained (Nader et al., 2014). While no substantial basal changes in 45S pre-rRNA expression were observed in the present study, the change in 45S pre-rRNA levels between PRE and POST was greater for both concurrent training groups compared with RT performed alone. Concurrent exercise also increased 45S pre-rRNA levels at 3 h post-exercise, with little effect of single-mode RT. These observations may be explained by the muscle sampling timepoints employed in the present study. Increased post-exercise 45S pre-rRNA levels have been previously shown at a later timepoint of 4 h after RE (Nader et al., 2014), whereas a reduction in 45S rRNA levels has been demonstrated 1 h post-RE in trained, but not untrained, states (Figueiredo et al., 2015). The possibility therefore exists that RT may increase 45S rRNA expression at a later timepoint post-exercise, and the sampling time points employed herein were not extensive enough to measure any exercise-induced increases in 45S pre-rRNA expression.

The effects of training on the basal expression of mature ribosome species 5.8S, 18S, and 28S were also investigated, as well as early post-exercise changes in mature rRNA expression. Contrary to the a-priori hypothesis, RT decreased levels of both the 5.8S and 28S rRNAs in the basal state post-training, while the training-induced change in both of these rRNAs was greater with concurrent exercise compared with RT alone. Neither training protocol was observed to induce any changes in 18S rRNA expression. Previous work in humans (Figueiredo et al., 2015) has observed basal increases in 5.8S, 18S, and 28S rRNA expression after 8 weeks of RT, all of which were reduced 1 h following a single bout of RT performed post-training. The present data contrast these findings by suggesting that in parallel with training-induced changes in total RNA content, RT performed alone was an insufficient stimulus to increase mature rRNA content, whereas concurrent exercise was sufficient to increase mature 5.8S and 28S expression after a single post-training exercise bout.

The rRNA primers used in the present study were specifically designed (Figueiredo et al., 2015) to differentiate between mature rRNA expression and the expression of these sequences still bound the polycistrionic 45S rRNA precursor. Unlike previous work (Figueiredo et al., 2015) showing mature rRNA expression was not associated with likewise increased expression of transcripts still bound to the 45S precursor, expression of these transcripts (5.8S, 18S and 28S [span]) were similarly increased in the present study. The authors explained their observation of simultaneous increases in 45S pre-rRNA and mature rRNA species by the rapid processing of 45S into its constituent rRNAs (Figueiredo et al., 2015). Given we observed similarly increased expression of both mature rRNA transcripts and those still bound to the 45S pre-rRNA (i.e., 'span' transcripts), our data suggests the observed changes in these markers may be reflective solely of changes in 45S pre-rRNA content and not mature forms of these rRNAs. These observations may also relate to the post-exercise time courses examined in the present study. In support of this notion, it was shown that a single bout of RE was sufficient to increase only the expression of rRNA transcripts still bound to the 45S pre-rRNA, and not mature rRNA species, even after 48 h of post-exercise recovery (Figueiredo et al., 2016b). It is therefore plausible that the post-exercise time courses examined in the present study were not extensive enough to measure early post-exercise changes in mature rRNA expression. Clearly, further work is required to investigate the time course of rRNA regulation with training in human skeletal muscle.

Previous work in both rodent (Goodman et al., 2011b; Miyazaki et al., 2011; Nakada et al., 2016; von Walden et al., 2012) and human (Figueiredo et al., 2015; Haddad et al., 2005) skeletal muscle have shown increases in total RNA content in parallel with load-induced muscle hypertrophy. Training-induced changes in total RNA content of skeletal muscle was also shown to be correlated (r = 0.72) with increased whole-muscle CSA (measured via CT) in humans after eight weeks of RT (Figueiredo et al., 2015). Consistent with the increased expression of mature 5.8S and 28S rRNA species, we observed increases in total RNA content for the HIT+RT group. Rather than increase RNA content as anticipated, a basal reduction in total RNA content was observed with RT performed alone. Despite this paradoxical finding, it is interesting to note total RNA content was substantially higher at PRE for the RT group compared with both the HIT+RT and MICT groups (1.8- and 1.6-fold, respectively). The reason for this between-group discrepancy at baseline is not immediately clear, given that data presented in Chapter 4 suggests these training groups had no differences in baseline lean mass measured via DXA or lower-body 1-RM strength. It is possible the training program employed in the present study was simply an insufficient stimulus to at least maintain this elevated basal RNA content for the RT group. Studies demonstrating robust increases in total RNA content concomitantly with rodent skeletal muscle hypertrophy typically employ supraphysiological methods for inducing muscle hypertrophy, such as synergist ablation (Goodman et al., 2011b; Miyazaki et al., 2011; Nakada et al., 2016; von Walden et al., 2012), a stimulus clearly not replicated by RT in human models. Participant training status may also impact upon training-induced changes in ribosome biogenesis in humans. The participants in the study by Figueiredo et al. (2015) were likely untrained (although this was not explicitly made clear) and were asked to refrain from RT for 3 weeks prior to the study. The participants in the present study were actively engaging in resistance and/or endurance exercise for at least 1 year prior to commencing the study, likely suggesting a higher relative training status compared with those of Figueiredo et al. (2015). Also supporting the notion of RT in the present study being an insufficient stimulus for ribosome biogenesis is that the training-induced change in total RNA was negatively correlated (r = -0.885, $P < 0.001$) with baseline total RNA content. This suggests individuals with a lower RNA content at PRE tended to increased total skeletal muscle RNA content with training, whereas individuals with a high pre-training RNA content tended to decrease total RNA content following training. It is possible that between-group differences in training volume,

which was clearly higher for the concurrent training groups compared with RT, may have impacted upon the training-induced changes in total skeletal muscle RNA content. Moreover, it is unclear whether those in the RT group had higher recreational training volumes prior to commencing the study, which may have been reduced upon commencing the study, subsequently resulting in a reduced total skeletal muscle RNA content after the intervention. Nevertheless, RT was sufficient to increase type I muscle fibre CSA, and to a similar extent type II fibre CSA (ES, 0.10 and 0.09, respectively) although this change was not substantial in magnitude nor statistically significant. In agreement with previous research (Bell et al., 2000; Kraemer et al., 1995), the training-induced increase in type I muscle fibre CSA was attenuated with concurrent exercise, albeit only when incorporating HIT, compared with RT performed alone. Despite these between-group differences in fibre-type specific hypertrophy, we could find no evidence that changes in lean mass or muscle fibre CSA were correlated with changes in total RNA content of skeletal muscle. We did, however, note relationships between total RNA content at POST and both type I ($r = 0.591$) and type II ($r = 0.470$) muscle fibre CSA at POST, as well the training-induced changes in both the CSA of type I muscle fibres ($r = 0.454$) and lower body lean mass ($r = -0.600$) measured via DXA. The apparent disconnect between training-induced changes in total RNA content and markers of muscle hypertrophy both at the whole-body and muscle-fibre levels suggests further investigation is required into relationship between changes in translational capacity and RT-induced hypertrophy in human skeletal muscle.

As skeletal muscle mass accretion is ultimately determined by the net balance between MPS and protein degradation (Atherton & Smith, 2012), the expression of ubiquitin ligases purported to mediate muscle protein breakdown (Bodine et al., 2001a) was also measured as proxy markers of protein degradation. Concurrent exercise incorporating HIT has previously been shown to exacerbate the expression of MuRF-1 relative to RT performed alone (Apro et al., 2015), while similar increases in MuRF-1 mRNA expression 3 h after a single bout of concurrent exercise incorporating either HIT or MICT in relatively training-unaccustomed individuals were reported in Chapter 3. Conversely, when performed in the trained state, the present data suggest only the HIT protocol was sufficient to induce elevated MuRF-1 expression after subsequent RT, relative to RT either performed alone or in combination with MICT. While the role of Atrogin-1 in mediating protein degradation is less clear compared with MuRF-1 (Krawiec et al., 2005), we nevertheless observed a reduction in Atrogin-1 expression at

+3 h for RT, but not for either concurrent training group. These data are consistent with previous reports of reduced Atrogin-1 expression 3 h after RT performed in both untrained and trained states (Fernandez-Gonzalo et al., 2013), but contrast others showing reduced Atrogin-1 expression 3 h after RT only when preceded 6 h earlier by MICT (40 min cycling at 70% of peak power output) (Lundberg et al., 2012). Taken together, these data suggest concurrent exercise incorporating HIT may exacerbate post-exercise rates of protein degradation by increasing MuRF-1 mRNA expression, while both concurrent exercise protocols prevented the acute reduction in Atrogin-1 expression induced by RT alone. These data should, however, be considered with recent evidence suggesting increased rates of protein degradation may be necessary to promote skeletal muscle remodelling and be permissive, rather than inhibitory, for training adaptations in skeletal muscle (Vainshtein & Hood, 2015).

Contrary to data presented in Chapter 3, performing HIT in combination with RT was a potent stimulus for upregulating PGC-1α mRNA expression, compared when RT was combined with MICT or performed alone. Traditionally implicated in the regulation of mitochondrial biogenesis via interactions with the nuclear- and mitochondrial-specific transcription factors (Pilegaard et al., 2003), additional roles of PGC-1α splice variants in promoting divergent skeletal muscle adaptation, including RE-induced hypertrophy, have emerged (Ruas et al., 2012). Despite these associations, other recent studies have questioned both the mode-specificity (Ydfors et al., 2013) and relationship between PGC-1α splice variant expression and RT-induced hypertrophy (Lundberg et al., 2014a). While the PGC-1α primers used in the current study cannot distinguish between PGC-1α isoforms, we did not observed a basal post-training reduction in PGC-1α expression for RT, and no changes in PGC-1α expression following a single bout of RT performed post-training. These data indicate this PGC-1α isoform is preferentially induced by endurance exercise, and that HIT is a particularly potent stimulus for increasing PGC-1α expression, even when performed in a training-accustomed state.

5.5.1 Conclusions

This is the first study to simultaneously investigate markers of ribosome biogenesis and mTORC1 signalling in human skeletal muscle following concurrent training compared with single-mode RT. Contrary to the presented hypotheses and

recent observations in humans (Figueiredo et al., 2015; Nader et al., 2014), little evidence of increased ribosome biogenesis was observed in skeletal muscle following eight weeks of RT. Rather, markers of ribosome biogenesis appeared to be greater following concurrent exercise, regardless of the endurance training intensity. This occurred despite a single bout of RT being a more potent stimulus for both mTORC1 signalling and phosphorylation of regulators of RNA Pol-1-mediated rDNA transcription (i.e., TIF-1A and UBF) when performed post-training. An apparent disconnect was noted between training-induced changes in muscle fibre CSA, of which the small increases induced by RT were attenuated for HIT+RT, and total skeletal muscle RNA content. Overall, the present data suggest single-mode RT performed in a training-accustomed state preferentially induces mTORC1 and ribosome biogenesis-related signalling in skeletal muscle compared with concurrent exercise; however, this is not associated with basal post-training increases in markers of ribosome biogenesis. As these responses were measured post-training, this may suggest RT may become a greater stimulus for ribosome biogenesis and muscle hypertrophy if training were continued long-term. Further work in human exercise models which stimulate more robust skeletal muscle hypertrophy (e.g., high-volume RT performed to failure), together with longer intervention periods, may be required to fully elucidate the role of ribosome biogenesis in adaptation to RT and subsequently any potential interference to these responses with concurrent training.

Chapter 6. General discussion and conclusions

The overall aim of this thesis was to determine the potential role of endurance training intensity in modulating the interference effect during concurrent training. Interference was investigated in the form of both i) molecular responses and adaptations in human skeletal muscle purported to regulate muscle mass, and ii) changes in maximal strength, CMJ performance, lean mass and muscle fibre hypertrophy, after concurrent training compared with single-mode RT. Chapter 3 (Study 1) of this thesis examined perturbations in mTORC1 signalling and the expression of microRNA species following single bouts of concurrent exercise incorporating either HIT or MICT as the endurance exercise modality. Chapters 4 and 5 (Study 2) of this thesis then explored the effects of eight weeks of concurrent training, incorporating either HIT or MICT, on exercise performance, morphological and molecular adaptations in human skeletal muscle, compared with RT performed alone. The following section will summarise the main findings from each of the chapters presented within this thesis, discuss both the inherent limitations and practical implications of these findings, before finally presenting recommendations for future research in the area of interference during concurrent training.

6.1 Summary of key findings

- Compared with RE performed alone, a single bout of either HIT or work-matched MICT performed prior to RE does not compromise skeletal muscle mTORC1 signalling (i.e., mTOR and p70S6K1 phosphorylation) in the 3 h post-exercise recovery period. Rather, combining RE with HIT was a particularly potent stimulus for increasing post-exercise mTOR and rps6 phosphorylation, and for reducing eEF2 phosphorylation and the expression of candidate microRNAs implicated in the negative regulation of the IGF-1/Akt, Fox-O1 and myogenesis pathways in skeletal muscle. These responses occurred despite similar metabolic perturbation (i.e., muscle glycogen depletion and markers of increased AMPK activity) induced in skeletal muscle by prior HIT or MICT before subsequent RE was commenced.

- Compared with RT performed alone, eight weeks of concurrent training incorporating either HIT or work-matched MICT cycling similarly attenuated

maximal (1-RM) lower-, but not upper-body strength development. Increases in lower-body lean mass were attenuated with concurrent training incorporating HIT, but not MICT. The attenuated lean mass responses for the HIT+RT group were also reflected by an attenuation of training-induced increases in *vastus lateralis* type I muscle fibre CSA compared with RT performed alone. Concurrent training also compromised training-induced improvements in selected CMJ variables, including peak force and power. These data corroborate existing evidence that endurance training can interfere with selected adaptations to RT, however extend current knowledge that endurance training intensity appears to not mediate interference to maximal strength gain, at least on a work-matched basis, and that HIT may to some extent negate lean mass gain during concurrent training.

- When performed in a training-accustomed state, a single bout of RE was a more potent stimulus for inducing both mTORC1 signalling and the phosphorylation of upstream regulators of RNA Pol-I-mediated transcription of the 45S rRNA precursor in human skeletal muscle, compared with concurrent exercise. In contrast to previous studies in relatively training-unaccustomed individuals (Carrithers et al., 2007; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015), these data provide evidence of interference to mTORC1 and ribosome biogenesis signalling following a single bout of concurrent exercise relative to RE performed alone. These observations affirm the need to measure early post-exercise molecular responses to concurrent exercise in a training-accustomed state, which likely streamlines their mode-specificity (Coffey et al., 2006a; Coffey et al., 2006b; Wilkinson et al., 2008).

- Despite the findings regarding the potency for single-bout RE in promoting mTORC1 and ribosome biogenesis signalling, and contrary to previous human studies (Figueiredo et al., 2015; Nader et al., 2014), no substantial evidence of ribosome biogenesis adaptation was observed in human skeletal muscle following RT performed alone, while these responses appeared greater following concurrent training. Concurrent training therefore did not appear to

attenuate any hypothesised RT-mediated increases in ribosome biogenesis and translational capacity of skeletal muscle, at least after eight weeks of training. There were also no substantial relationships between training-induced changes in markers of muscle hypertrophy and total skeletal muscle RNA content, contrasting previous human data (Figueiredo et al., 2015), although post-training total RNA content was related to both type I (r = 0.591) and type II (r = 0.470) muscle fibre CSA, and also to the training-induced change in both type I muscle fibre CSA (r = 0.454) and lower-body lean mass (r = -0.600). Further work utilising interventions inducing more robust muscle hypertrophy responses and longer training periods (see section 6.4.2) may be required to fully elucidate the role of altered regulation of translational efficiency and capacity in mediating any potential interference effect during concurrent training.

This thesis extends current knowledge on the role of endurance training intensity in altering early post-exercise molecular responses to concurrent exercise in human skeletal muscle, and interference to RT adaptations following short-term concurrent training. Previous single-bout concurrent training investigations have incorporated either HIT (Apro et al., 2015; Pugh et al., 2015) or MICT (Apro et al., 2013; Carrithers et al., 2007; Donges et al., 2012; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012) when attempting to elucidate whether interference to mTORC1 signalling might explain long-term interference to RT adaptations. To date, none of these studies have demonstrated any evidence of interference to mTORC1 signalling or protein synthesis as seen in rodent skeletal muscle following *ex-vivo* electrical stimulation (Atherton et al., 2005). The data presented within this thesis further questions whether interference to mTORC1 signalling plays a role in the interference effect; however, these data also suggest the mTORC1 pathway is preferentially induced in skeletal muscle by RE compared with concurrent exercise when performed in a training-accustomed state. Regarding the potential role of endurance training intensity in promoting post-exercise to post-exercise anabolic responses in skeletal muscle, combining RE with HIT, compared to with MICT, was a more potent stimulus for inducing both anabolic (i.e., mTORC1 signalling) and catabolic (i.e., MuRF1 and FoxO1 mRNA expression) responses, when performed in a relatively training-unaccustomed state. The present data also report for the first time that concurrent training incorporating HIT, but not MICT, reduces the expression of candidate miRNAs purported to negatively regulate signalling

pathways involved in skeletal muscle hypertrophy and/or myogenesis. Thus, rather than promoting interference, we observed evidence concurrent training incorporating HIT appears to be a potent stimulus for promoting a skeletal muscle milieu conducive to promoting positive skeletal muscle adaptations to training.

Despite the observations of the impact of concurrent vs. single-mode RE on post-exercise molecular responses in skeletal muscle, it has become increasingly clear single-bout exercise studies cannot 'predict' chronic phenotypes induced by long-term training (Mitchell et al., 2014; Phillips et al., 2013; Wilkinson et al., 2008). The aim of Chapter 4 of this thesis was therefore to elucidate the effects of 8 weeks of concurrent training incorporating either HIT or MICT on adaptations to maximal strength, CMJ performance, body composition, and aerobic capacity, relative to single-mode RT. The findings from this study confirmed concurrent endurance training can interfere with selected adaptations to RT, albeit to differing degrees, but extend current knowledge on the role of endurance training intensity in mediating this interference effect. Contrary to the hypotheses, a similar degree of interference to maximal strength development was noted with concurrent training regardless of whether HIT or work-matched MICT was incorporated, suggesting endurance training volume may be a more critical variable in mediating this phenomenon. This is consistent with the conclusions from a meta-analysis investigating the contribution of concurrent training variables to the interference effect (Wilson et al., 2012), and a study whereby interference to maximal strength was only noted with higher- compared with lower endurance training volumes (Jones et al., 2013). Another novel finding from this thesis was performing HIT concurrently with RT resulted in less lower-body lean mass gain when compared with RT either performed alone or in combination with MICT, for which lower-body lean mass similarly improved. Considering the potency of a single bout of concurrent exercise incorporating HIT for inducing mTORC1 signalling and reducing miRNA expression in skeletal muscle (Chapter 3), this highlights the disconnect between early post-exercise responses in skeletal muscle and longer-term training adaptations. Another consideration when interpreting the outcomes of Chapter 4 are potential between-group differences in total training volume when considering training completed external to the study, a concept largely ignored in the literature. Using the session RPE (sRPE) method, an attempt was made to quantify any potential between-group differences in the volume of training completed external to the study, and noted participants in the HIT+RT group had a higher internal training load than those in the

MICT+RT group. It is therefore difficult to deduce whether this effect was mediated via between-group divergences in endurance training intensity or total endurance training volume *per se*. There is a clear need for future studies in this area to further clarify the potential role of endurance training volume in promoting interference by manipulating between-group endurance training volumes (see section 6.4).

The final chapter of this thesis (Chapter 5) investigated additional novel molecular mechanisms that may contribute to interference to muscle hypertrophy and strength with concurrent training compared with single-mode RT. There is growing evidence that adaptations to translational capacity in skeletal muscle occur concomitantly with skeletal muscle hypertrophy (Chaillou et al., 2014; Figueiredo et al., 2015; Nader et al., 2014) and appear attenuated in situations where muscle mass is likewise compromised (Figueiredo et al., 2016a; Figueiredo et al., 2016b; Kirby et al., 2015). In this context, it was investigated whether adaptations to translational capacity might be differentially affected by concurrent training, and whether any evidence of molecular interference would be observed following a single bout of exercise performed in a training-accustomed state, in contrast to Chapter 3. Contrary to our hypothesis, however, we did not observe any evidence of substantial ribosome biogenesis adaptation to RT performed alone, while training-induced changes in markers of ribosome biogenesis were greater with concurrent training. These changes occurred despite a single bout of RE being a more potent stimulus for inducing mTORC1 signalling and the phosphorylation of upstream regulators of RNA Pol-I-mediated rDNA transcription (i.e., TIF-1A and UBF) when performed post-training. It is unclear, however, whether RT may have become a more potent stimulus for inducing ribosome biogenesis adaptation with an extended training period (>8 weeks). The possibility exists that the RT intervention employed was simply an insufficient stimulus for promoting ribosome biogenesis adaptation in skeletal muscle, when compared to supraphysiological rodent models of inducing skeletal muscle hypertrophy such as synergist ablation (Goodman et al., 2011b; Miyazaki et al., 2011; Nakada et al., 2016; von Walden et al., 2012), and considering the participants were recreationally active and not untrained. In agreement with previous research (Bell et al., 2000; Kraemer et al., 1995), the training-induced increase in type I muscle fibre CSA was attenuated with concurrent exercise, albeit only when incorporating HIT, compared with RT performed alone. Despite these between-group differences, we could find no evidence that changes in lean mass or muscle fibre CSA were correlated with changes in total RNA content of

skeletal muscle. Taken together, it appears that divergences in skeletal muscle responses related to both translational efficiency (i.e., enhanced mTORC1 signalling) and translational capacity (i.e., ribosome biogenesis) do not fully explain the phenotypes induced by 8 weeks of concurrent training. The apparent disconnect between total RNA content and markers of muscle hypertrophy both at the whole-body and muscle-fibre levels suggests further investigation is required into relationship between changes in translational capacity and lean mass gain.

To summarise, this thesis has contributed new knowledge to the field of concurrent training by investigating both the role of endurance training intensity in promoting interference to RT adaptations, as well as novel molecular mechanisms that may contribute to the interference phenomenon. The findings of this thesis suggest endurance training intensity *per se* may play a limited role in promoting interference during concurrent training, and the likely greater contribution of endurance training volume warrants further investigation. When performed in a relatively training-unaccustomed state, combining HIT with RT is a potent stimulus for inducing mTORC1 signalling and reducing the expression of miRNA species purported to be involved with skeletal muscle adaptation to exercise. In contrast, performing RT in a training-accustomed state preferentially enhances both mTORC1 and ribosome biogenesis signalling when compared with concurrent exercise. Despite concurrent exercise incorporating HIT being a potent stimulus for anabolic responses in skeletal muscle when performed in a relatively training-unaccustomed state, attenuated markers of training-induced muscle hypertrophy were observed when HIT was incorporated into 8 weeks of concurrent training. It is clear, therefore, that early post-exercise molecular responses do not appear to explain interference to RT adaptations, at least after 8 weeks of training. Given the between-group discrepancies in interference to training-induced maximal strength gain and indices of muscle hypertrophy observed in Chapters 4 and 5, the potential exists that the majority of interference to maximal strength gain is largely mediated by non-hypertrophic (i.e., neural) mechanisms known to predominate in the early weeks of RT (Folland & Williams, 2007; Gabriel et al., 2006; Moritani & deVries, 1979). It is unclear whether longer training periods, which are likely to induce more substantial muscle hypertrophy (Folland & Williams, 2007; Gabriel et al., 2006; Moritani & deVries, 1979), are required to provide further insight into the factors mediating interference specifically to muscle hypertrophy responses following longer-term concurrent training.

6.2 Limitations and considerations

The primary findings presented above and within the chapters of this thesis should be considered in context with the limitations of the methodological and analytical techniques employed, of which a brief discussion is presented below.

- Caution should be taken when interpreting between-condition differences in post-exercise molecular responses as markers of future training adaptations. As reported in similar studies investigating molecular interference in skeletal muscle with concurrent training (Apro et al., 2013; Coffey et al., 2009a; Coffey et al., 2009b; Fernandez-Gonzalo et al., 2013; Lundberg et al., 2012; Lundberg et al., 2014b; Pugh et al., 2015), we used the phosphorylation of components of the mTORC1 pathway as proxy markers for the activation of protein synthesis. However, a direct coupling between mTORC1 signalling and protein synthesis rates does not always exist (Atherton et al., 2010), nor does greater phosphorylation of mTOR pathway intermediates always equal a greater protein synthetic response (Crozier et al., 2005). Even functional measures such as rates of MPS after a single bout of RE do not correlate with muscle hypertrophy after 16 weeks of RT (Mitchell et al., 2014). Adding complexity to the relationship between molecular responses in skeletal muscle and chronic training adaptations is the transient and time-course dependent nature of these responses post-exercise (Drummond et al., 2011; Wojtaszewski et al., 2003). As a consequence, the relative magnitude of these responses is influenced by the post-exercise biopsy sampling timepoints employed in any given study. It is likely that employing different post-exercise muscle sampling time points to those investigated in the present thesis would have influenced the relative magnitude of the measured responses, and therefore any associated inferences on these data. Further work is required to define the time course of molecular events mediating chronic phenotypic adaptations to training, and potentially their progression during a training program. Such information is likely to also provide further mechanistic insight into the concurrent interference effect (Fyfe et al., 2014).

- The potential for muscle fibre type-specificity in the molecular responses and adaptations measured in skeletal muscle must be considered. Muscle analyses for

studies comprising Chapters 3 and 5 were conducted using mixed whole-muscle homogenate. However, this approach may have masked any fibre-type specific differences in measured responses following the exercise protocols, including glycogen content, signalling responses and gene expression (Murphy & Lamb, 2013). For example, although the HIT and MICT protocols induced similar whole-muscle glycogen depletion and markers of AMPK activity in the study presented in Chapter 3, there would likely be fibre-type specific differences in these responses (Suriano et al., 2010). Thus, whether the prior endurance exercise protocols altered signalling responses and/or gene expression in a fibre-type specific manner following subsequent RE remains to be determined.

- The use of DXA as a measure of whole-body changes in body composition is not without limitations. DXA is advantageous as a measure of whole-body changes in body composition, and scans may also be segmented into separate body portions by the investigator, allowing for differentiation in body composition changes between the upper- and lower-body, for example, as reported in Chapter 4. While DXA is a useful measure of overall body composition, its local resolution is lower than MRI (magnetic resonance imaging) (Maden-Wilkinson et al., 2013), which may have provided a better measures of region-specific muscle hypertrophy; however, these measures were unfortunately not possible within this thesis. It is also possible that the training-induced changes in lean mass reported in Chapter 4 may have been underestimated due to the insensitivity of DXA-derived lean mass measurements.

- The mechanism(s) underlying the interference effect may involve either i) an exacerbation of residual fatigue after endurance exercise, which compromises RE quality and subsequently adaptation, or ii) a molecular mechanism(s) whereby post-exercise molecular responses initiated by endurance exercise antagonise those mediating increased MPS, and subsequently hypertrophy, consequent to RE (see section 2.9). The observations of attenuated maximal strength gain in Chapter 4, in the absence of a similar magnitude of interference to muscle hypertrophy or adaptations to translational capacity of skeletal muscle, suggest these underlying mechanisms may be non-hypertrophic and potentially neural in origin. Indeed, it is well established that neural adaptations predominate in the early phases of strength

training, whereas robust muscle hypertrophy later ensues (Folland & Williams, 2007; Gabriel et al., 2006; Moritani & deVries, 1979). The possibility exists that most concurrent training studies, including those presented within this thesis, are only long enough in duration to detect interference mediated predominantly by neural factors. Despite evidence of muscle hypertrophy occurring after just 3 weeks of RT (DeFreitas et al., 2011; Seynnes et al., 2007), it is likely that interference to processes underpinning muscle hypertrophy will become more apparent following longer-term (i.e., months to years) training.

- There are a multitude of potential training variables associated with concurrent training (i.e., endurance and RT volume, intensity, and modality, training frequency, order of resistance and endurance training and between-mode recovery), further discussed in Fyfe et al. (2014). The studies conducted within this thesis focused solely on the manipulation of endurance training intensity during concurrent training, while attempting to control for the influence of other potential confounding variables (e.g., training volume, resistance and endurance training order, between-mode recovery and endurance training modality). It is possible the results presented within this thesis may have differed if other training variables had been differentially manipulated. For example, both the exercise order (i.e., endurance training performed before RT or vice versa) and the length of between-mode recovery may influence the degree of residual fatigue and/or AMPK activity in which subsequent RT is commenced, subsequently modulating the degree of interference to RT adaptations. It is likely that both the exercise order (i.e., endurance training before RT) and length of between-mode recovery (i.e., 10-15 min) employed within the present thesis were sub-optimal for promoting adaptation to subsequent RT compared to the alternate exercise order and longer between-mode recovery periods. The outcomes of this thesis should therefore be considered in context of the possibility that endurance training intensity may play a greater or lesser role in the interference effect if factors such as those mentioned above are differentially manipulated.

- For all studies conducted within this thesis, cycling was employed as the endurance training modality. While cycle ergometry provides a number of practical advantages

for the researcher, including ease of workload quantification and availability of equipment, the results of these studies may not be generalised to other exercise modalities, in particular running exercise. There is accumulating evidence that running exercise may exacerbate interference to RT adaptations during concurrent training, compared to when cycling is employed (Leveritt et al., 1999; Wilson et al., 2012), although randomised controlled trials have not yet been conducted to fully elucidate any modality-dependent interference effect. Further work is required to determine the relative contribution of endurance training modality to interference; however, the findings of this thesis apply to concurrent training regimes incorporating cycling exercise.

- As discussed in section 5.5, individual responses appear to be evident in the training-induced changes in maximal strength, hypertrophy, and CMJ variables presented in Chapter 4 (Figure 4.4). The possibility exists that some individuals may also be more or less susceptible to interference to RT adaptations with concurrent training compared with other individuals. To adequately evaluate individual responses to a controlled trial such as that conducted in Chapter 4, however, large sample sizes and repeated measurements of outcome measures are necessary (Hopkins, 2015), criteria in which studies within this thesis were unfortunately lacking. Future studies in this area should consider employing designs whereby inferences on the degree of individual response to concurrent training, and potentially interference to RT adaptations, may be appropriately evaluated. Such information may aid in the development of individualised exercise prescription guidelines to minimise the interference effect during periods of concurrent training.

6.3 Practical applications

The results gleaned from this thesis may inform practical recommendations for simultaneously maximising adaptation to both resistance and endurance exercise during concurrent training. These recommendations are briefly summarised below:

- The conclusions of Chapter 4 affirm previous findings that performing endurance training concurrently with RE indeed results in an interference effect to

improvements in maximal strength, CMJ performance, and to a lesser extent lean mass development. However, there were no substantial differences in either of these outcomes between concurrent training groups incorporating either HIT or work-matched MICT as the endurance training modality. Taken together, these data suggest endurance training intensity is not a critical mediator of interference to maximal strength and peak CMJ force and power, at least after 8 weeks of training, and total endurance training volume may be more critical. Thus, during periods of concurrent training it is suggested endurance training volumes be limited so as to minimise any volume-dependent interference effect. Whether low-volume HIT protocols, previously shown to cause no interference to maximal strength development with concurrent endurance and RT performed on separate days (Cantrell et al., 2014), confer advantage by limiting interference compared with higher-volume HIT or MICT protocols, remains to be elucidated.

- The effects of endurance training intensity on lean mass gain consequent to concurrent training require further investigation. The trend for compromised DXA-measured lean mass responses for the HIT+RT group was also reflected by an attenuated increase in training-induced type I muscle fibre CSA. An important consideration, however, is we also noted a higher non-prescribed internal training load for the HIT+RT group, suggesting total endurance training volume during the intervention period may have been higher for this group. Further work is therefore required to determine the potential influence of endurance training intensity and/or volume on interference to lean mass gain before practical recommendations can be made. The potential impact of training prescription and nutrient availability on training-induced lean mass gain is further discussed in the following section (6.4), and are additional considerations when interpreting these results.

6.4 Recommendations for future research

The following recommendations are presented for future research investigating the interference phenomenon with concurrent training:

Single-bout exercise studies should be conducted in a training-accustomed state

- The combined observations of molecular responses in skeletal muscle after single exercise bouts presented in both Chapters 3 and 5 of this thesis are consistent with the notion that post-exercise molecular signals are rather exercise mode-unspecific in relatively untrained skeletal muscle (Camera et al., 2010; Coffey et al., 2006b; Vissing et al., 2011; Wilkinson et al., 2008). When considered together with the limited relationship between molecular signalling responses and/or rates of MPS following early exercise bouts and chronic training adaptations (Mitchell et al., 2014), future studies should, where possible, conduct long-term (>12 weeks) training studies and measure post-exercise skeletal muscle responses in a training-accustomed state. However, further research is required to determine whether early molecular responses in trained muscle are more predictive of phenotypes induced by further training.

Training interventions should be sufficient to induce considerable muscle hypertrophy

- For studies investigating interference to RT adaptations during concurrent training, it is imperative the intervention is sufficient to induce robust skeletal muscle hypertrophy. Key variables in maximising skeletal muscle hypertrophy appear to be sufficient RT volume (Burd et al., 2010), training closer to the point of concentric failure (even with low relative training loads) (Mitchell et al., 2012), length of the training intervention, as well as amino acid availability (Cermak et al., 2012). Future studies in this area should attempt to adhere to these prescription guidelines for maximising hypertrophy, as well as providing sufficient dietary control/support during the training intervention (Cermak et al., 2012). Implementing these measures may improve the likelihood of future studies detecting any interference to skeletal muscle growth with concurrent training.

Additional mechanisms that may be involved with the interference effect warrant further investigation

- Studies conducted within this thesis have primarily investigated changes in translational efficiency (i.e., mTORC1 signalling) and translational capacity (i.e., ribosome biogenesis) as potential mechanisms explaining previous observations of

attenuated muscle hypertrophy and strength following concurrent training. However, additional mechanisms, such as satellite cell activation, are warranted of further investigation. Although the role of satellite cells in promoting skeletal muscle hypertrophy is controversial (O'Connor & Pavlath, 2007; Rehfeldt, 2007), concurrent training has previously been shown to attenuate early post-exercise satellite cell responses compared with single-mode RE (Babcock et al., 2012). Whether these responses are also attenuated following longer-term training is worthy of future investigation.

- The potential muscle fibre-type specificity of early post-exercise responses and long-term adaptations with concurrent training also requires further examination. The induction of mTORC1 signalling following RE has previously been shown to predominate in type II muscle fibres (Koopman et al., 2006), which are also most susceptible to RE-induced growth (Tesch, 1988). Whether examining post-exercise molecular responses in mixed skeletal muscle homogenate masks any potential fibre type-specificity in responses is therefore unclear, and should be a consideration for future research.

- Another novel aspect to the regulation of anabolic responses in skeletal muscle is the role of intracellular translocation of signalling proteins in response to anabolic stimuli, such as amino acid provision (Sancak et al., 2010) or mechanical loading (Jacobs et al., 2013). Given the important role of an mTOR-lysosomal association in promoting mTOR activation, including both the targeting of mTOR towards the lysosome (Sancak et al., 2010) and concomitant dissociation of negative regulators of mTORC1 away from the lysosome (Jacobs et al., 2013), future studies in this area should investigate whether these processes are differentially regulated following concurrent training compared with RT alone. Together, investigation of these additional mechanisms relating to skeletal muscle anabolism, and subsequently muscle growth, will help to shed further light on the potential mechanisms underlying interference to muscle hypertrophy and strength with concurrent training.

Chapter 7. Reference list

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Appendices

Appendix A: Information to participants form used for Study 1 (Chapter 3)

INFORMATION TO PARTICIPANTS INVOLVED IN RESEARCH

You are invited to participate

You are invited to participate in a research project entitled "The effect of endurance exercise intensity on the acute molecular responses induced by subsequent resistance exercise in recreationally active males".

This project is being conducted by Mr Jackson Fyfe as part of a PhD study at Victoria University under the supervision of Dr Nigel Stepto and Prof David Bishop from the College of Sport and Exercise Science.

Project explanation

Simultaneously incorporating both strength and endurance training into a training program is known as concurrent training. While this is a common training approach that combines the benefits of both exercise modes, concurrent training often results in less muscle mass, strength and power improvements compared to undertaking strength training alone. However, very little is known regarding the factors which worsen or reduce this interference effect and the mechanisms by which this occurs.

Recently, high-intensity interval training (HIT) has become a popular and time-efficient training approach for improving aspects of health and performance. However, very little is currently known regarding the effect of high-intensity endurance exercise on interference when performed concurrently with resistance exercise.

This project will investigate the effect of endurance exercise intensity (i.e., HIT) on interference of the anabolic responses to subsequent resistance exercise when performed in a concurrent manner.

You are eligible to participate in this study if you are:

- Male and aged between 18 and 35
- Recreationally active in BOTH resistance and endurance exercise (undertaking each exercise mode 1-2 times per week for more than one year)
- Free from any current muscle or ligament injury of the lower body
- Free from any current or previous cardiovascular/respiratory condition or abnormality (e.g., heart rhythm disturbance, elevated blood pressure, diabetes)

What will I be asked to do?

You will firstly be asked to fill out several short questionnaires about your family medical history and exercise habits to assess your eligibility to participate in this study.

After confirming your eligibility for this study, you will undertake the following procedures, all conducted in Building P, Victoria University Footscray park campus:

1. *Familiarisation session*

You will be required to attend initial familiarisation session (~1 hour), during which you will be familiarised with all equipment and protocols to be performed in the preliminary testing and experimental trial sessions.

2. *Preliminary testing session*

Approximately 2-4 days after the familiarisation, you will undertake preliminary testing session sessions, during which you will undertake a graded exercise test (GXT) to determine your maximal aerobic capacity (VO_{2max}), lactate threshold (LT) and peak power (W_{max}) (~1 hour in duration), and a one repetition maximum (1-RM) single leg press test to determine your maximal lower body strength (~30 mins).

The GXT is an incremental exercise test consisting of multiple 4-minute work stages at increasing workloads, separated by 30 seconds recovery. The test will be terminated when you can no longer complete the desired workload. In order to measure lactate threshold, venous blood samples (~2 mL) will be obtained at rest, and immediately following completion of each work stage. After 5 minutes recovery, you will again cycle against a high workload until you cannot continue, and expired gases will be measured via a mouthpiece to calculate your maximal oxygen consumption (VO_{2max}).

The 1-RM test will be conducted on a seated leg press machine. After a standard warm-up, you will attempt to lift increasing loads until only one, but not a second, repetition is possible. Three minutes recovery will be allowed between 1-RM attempts, and testing will be completed on both legs separately.

3. *Experimental trials*

Approximately one week after the preliminary testing session, you will begin the first of three experimental trials (~ 5 hours each) each separated by at least one week.

An overview of the study design is provided in Figure 1 below:

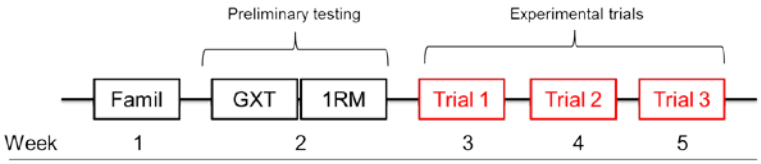


Figure 1. Study design overview

Upon arrival at the laboratory for each experimental trial, a plastic cannula will be placed into a forearm vein for blood sampling and you will then be provided with a standardised breakfast. After resting quietly for 45 min, you will then undertake either moderate intensity continuous (~30 min at 80% LT) or high-intensity interval cycling (10 x 2 min at 120% LT, 1 min passive recovery) for experimental trials 1 and 2. After resting quietly for 15 minutes, a muscle biopsy will be taken from an outer thigh muscle (i.e. the vastus lateralis) and you will then complete 8 sets of 5 single leg press repetitions at 80% 1-RM for each leg, with 3 minutes rest between sets. You will then rest quietly in the lab for 3 hours, during which additional muscle samples will be obtained 1 and 3 hours after resistance exercise. During this 3-hour recovery period you may watch movies or use a laptop quietly if you wish. Venous blood samples (~5 mL) will be drawn from the cannula after 0, 2, 5, 10 and 15 minutes recovery from endurance exercise, and 0, 2, 5, 10, 30, 60, 120 and 180 minutes after resistance exercise. For experimental trial 3 (RE only trial), you will undertake identical procedures but with the omission of any endurance exercise prior to the resistance exercise.

An overview of each experimental trial timeline is provided in Figure 2 below:

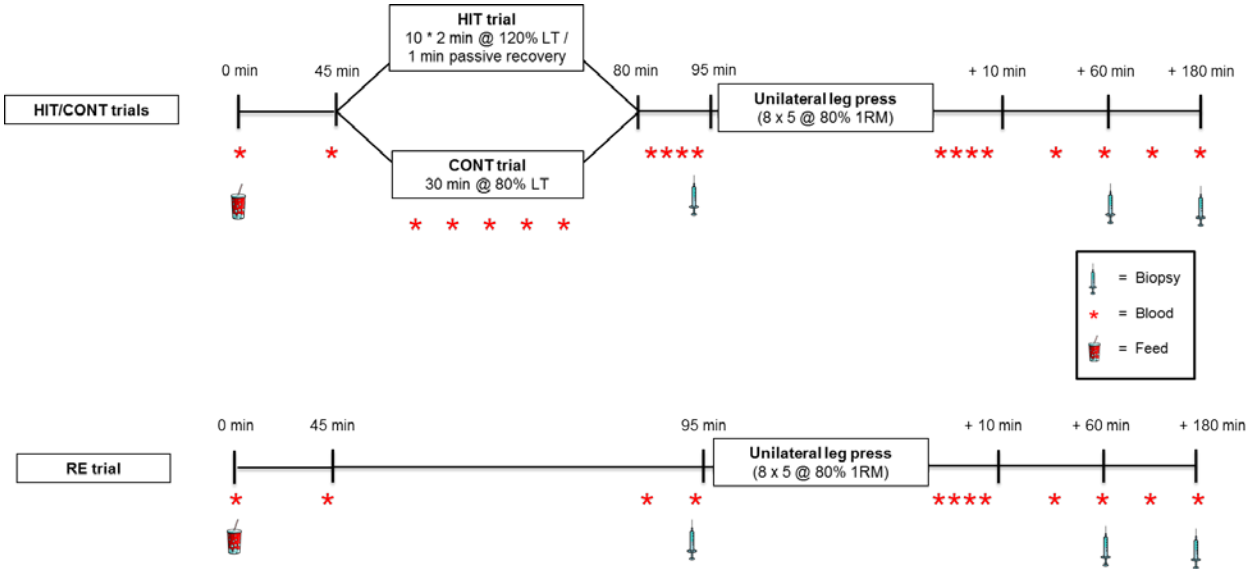


Figure 2. Experimental trial timelines

4. Exercise and diet control

You will be asked to refrain from any formal physical activity or training for at least 24 hours prior to each experimental trial. Additionally, you will be provided with a standardised diet that you will be required to strictly follow during the 24 hours prior to each experimental trial. On the morning of each experimental trial you will be required to report to the laboratory in a fasted state and we will provide you with a standardised breakfast upon your arrival.

The total time commitment for this study is approximately 17.5 hours across six separate visits.

What will I gain from participating?

While we cannot guarantee that you will gain any benefits from your participation in this study, you will however be provided with a $150 gift voucher after completing the study as compensation for your time. You will also receive potentially valuable information regarding your aerobic fitness and strength levels.

How will the information I give be used?

All data collected will be stored under alphanumeric codes (i.e., without your name or personal details) which will only be identifiable by the researchers. All muscle samples collected will be used to analyse some proteins and genes involved with adaptations to training. The data that will be collected during the study may be used in a thesis, at conference presentations and published in peer-reviewed scientific journals. All data will be presented anonymously so your confidentiality is maintained. With your written consent, photographs or videos may be taken during experimental trials for use in presentations or to assist in future experimental set-ups. Any images will only be taken with your written consent and in all cases you will be de-identified.

What are the potential risks of participating in this project?

The procedures involved in participating in this study are of low risk. Nevertheless, as in any invasive and exercise procedure, there are small risks and some discomfort that may be experienced:

Exercise testing

You will experience the fatigue associated with strenuous exercise, particularly during the GXT. Nevertheless, as in any physical activity, there is a very small possibility of injuries that include, but are not

restricted to; muscle, ligament or tendon damage, breathing irregularities and dizziness. There is a high probability that you might experience mild muscle soreness for 2-3 days following the 1-RM strength testing, however this will not be more severe than is typically experienced after unaccustomed resistance exercise. There is also a small risk of muscle, ligament or tendon injury during the 1-RM test. However, all protocols are commonly performed in exercise physiology laboratories and potential risks to participants have been minimised by employing appropriate warm-up procedures and researcher supervision.

Intravenous cannulation
Needle insertion into a vein is required for placement of a cannula into a forearm vein and you will feel minor to moderate discomfort as a result. However, the needle is quickly removed and only a flexible plastic tube remains in your vein for the duration of blood sampling (approximately 5 hours). When the cannula is removed, direct pressure will be applied to the area to reduce the chances of bruising. Cannulas are routinely placed into veins of participants in clinical research studies and in hospital patients. The risks of IV cannulation are low, but very occasionally significant bruising or infection can occur. The researchers are qualified and experienced in venous cannula placement and the use of aseptic techniques.

Muscle biopsy
The muscle biopsy will be performed by a qualified medical doctor who is experienced in taking muscle biopsies. Xylocaine, a local anaesthetic, will be injected at the site of the muscle biopsy (vastus laterialis – mid outer thigh). The anaesthetic may burn or sting when injected before the area becomes numb. After it becomes numb minimal or no discomfort will be experienced during the procedure. Only slight pressure or a slight "pulling" sensation will be felt. To extract the muscle biopsy a small incision needs to be made where the muscle biopsy needle will be inserted. After the incision has been made a Bergstrom needle will then be inserted to extract a small muscle sample (approx. 2 rice grains in size). Once the local anaesthetic has worn off and for the next day or two the area will likely feel like you have been "corked". You should not feel any discomfort after 2-3 days. In rare cases haematomas have been reported, although these symptoms typically disappear within a week. On very rare occasions, altered sensation (numbness or tingling) on the skin near the site of the biopsy has been reported; however this sensation disappears over a period of a few weeks to months.

Who is conducting this study?

To express your interest in participating, or further information regarding this research, please contact:

Student researcher
Mr Jackson Fyfe
Tel: 03 9919 4066
Mob: 0419 371 076
Email: jackson.fyfe@live.vu.edu.au

Any queries about your participation in this project may be directed to the Chief Investigator listed above.
If you have any queries or complaints about the way you have been treated, you may contact the Ethics Secretary, Victoria University Human Research Ethics Committee, Office for Research, Victoria University, PO Box 14428, Melbourne, VIC, 8001 or phone (03) 9919 4781.

Appendix B: Information to participants form used for Study 2 (Chapters 4 and 5)

INFORMATION TO PARTICIPANTS INVOLVED IN RESEARCH

You are invited to participate

You are invited to participate in a research project entitled "The effect of endurance training intensity on interference between concurrent resistance and endurance exercise in recreationally-active males".

This project is being conducted by Mr Jackson Fyfe as part of a PhD study at Victoria University under the supervision of Dr Nigel Stepto and Prof David Bishop from the College of Sport and Exercise Science.

Project explanation

Simultaneously incorporating both strength and endurance exercise into a training program is known as concurrent training. While this is a common training approach that combines the benefits of both exercise modes, concurrent training often results in less muscle mass and strength improvements compared to if only strength training were performed. However, at present there is very little known about the factors which worsen or reduce this 'interference effect'.

High-intensity interval training (HIT) has recently become a popular and time-efficient training approach for improving multiple aspects of health and performance. However, whether HIT is a favourable exercise strategy to use when trying to simultaneously improve strength and muscle mass (i.e., concurrent training) is unclear. Potentially, higher-intensity endurance exercise may interfere with the ability to perform, and respond to, subsequent resistance exercise.

This project will compare the effects of HIT versus lower-intensity, traditional continuous endurance exercise on the interference effect when performed concurrently with resistance exercise, compared to undertaking resistance exercise alone.

You are eligible to participate in this study if you are:

- Male and aged between 18 and 40
- Recreationally active in resistance and/or endurance exercise (undertaking either exercise mode 1-2 times per week for more than one year)
- Free from any current muscle or ligament injury of the lower body
- Free from any current or previous cardiovascular/respiratory condition or abnormality (e.g., heart rhythm disturbance, elevated blood pressure, diabetes)

What will I be asked to do?

You will firstly meet with the student researcher and asked to fill out several short questionnaires about your family medical history and exercise habits, to assess your eligibility to participate in this study.

After confirming your eligibility for this study, you will undertake the following procedures, all conducted in Building P at Victoria University Footscray park campus:

5. Familiarisation

You will be required to attend initial familiarisation session (~1 hour), during which you will be familiarised with all equipment and testing protocols to be performed in the preliminary testing sessions.

6. Preliminary fitness testing

You will then undertake preliminary testing across the next three sessions to evaluate various aspects of your fitness. The first session (~1 hour) involves a graded exercise test (GXT) performed on a cycle ergometer to determine your aerobic fitness. The second session (~1 hour) will involve 1-repetition maximal (1-RM) strength and power (jump) testing, and the third session will involve a DEXA scan (~15 min) to estimate your body composition.

- The graded exercise test (GXT) is an incremental exercise test consisting of multiple 4-minute work stages at increasing workloads, separated by 30 seconds recovery. The test will be terminated when you can no longer complete the desired workload. In order to measure lactate threshold, venous blood samples (~1 mL) will be obtained at rest, and immediately following completion of each work stage. After 5 minutes recovery, you will again cycle against a high resistance until you cannot continue any further and expired gases will be measured via a mouthpiece to measure your maximal oxygen consumption (VO_{2max}). Before starting the GXT and immediately afterwards, an extra blood sample (~9 mL) will be obtained for the purposes of investigating the effects of the exercise on immune function.

- The maximal strength (1-RM) testing will be conducted on a seated leg press machine and bench press. After a standard warm-up, you will attempt to lift increasing loads until only one, but not a second, repetition is possible. Three minutes recovery will be allowed between 1-RM attempts.

- The maximal power (jump) testing involves repeated body-weight jumps performed on a force plate to measure your ability to produce force and power. The protocol will involve 3 maximal jumps (i.e., as high as you can), separated by 3 minutes of recovery.

- A DEXA scan will be performed to estimate your body composition (i.e., lean mass vs. fat mass). This involves lying still on the DEXA scanner for approximately 7 minutes while the scanner passes over you to assess your body composition.

7. Training period

The week after completion of preliminary testing, you will begin the 8-week training program. You will be RANDOMLY allocated to one of three training groups: 1) high-intensity interval training and resistance training (HIT+RT), 2) lower-intensity continuous cycling and resistance training (CONT+RT) or 3) resistance training only (RT only). Training sessions will be performed three (3) times per week and consist of either HIT or CONT cycling followed by resistance training, or resistance training only, depending on which group you are allocated to.

The HIT+RT group will perform multiple 2-min intervals separated by 1 min of recovery. The CONT+RT group will perform lower-intensity continuous cycling with no intermittent recovery. An identical resistance exercise session will be performed 10-min following each cycling bout. The RT only group will not perform any cycling before the resistance exercise session.

Each training session will last between ~40–110 min depending on the group you are allocated to. Training sessions will typically be conducted between 6:00 to 9:00 AM, however this can vary depending on your availability. Immediately before the first training session only, a single resting muscle biopsy will obtained from an outer thigh muscle (described below).

An overview of the study design is provided in Figure 1 below:

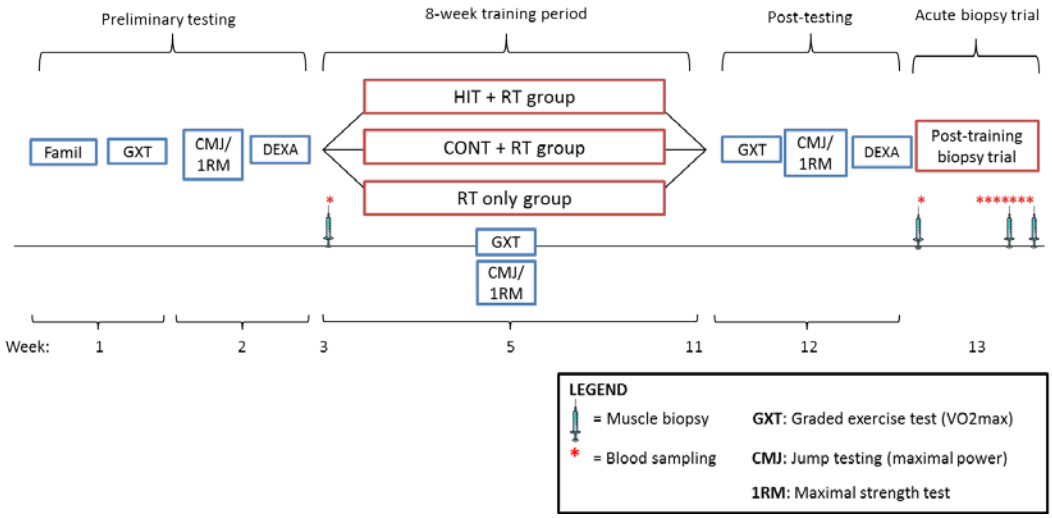


Figure 1. Study design overview

8. Mid- and post-training testing

Mid-way through the training program (in week 5), you will repeat the GXT and 1-RM testing so that all training intensities can be re-adjusted for the remainder of the training program. After completion of the training program, you will repeat all preliminary testing to evaluate how you have responded to the training program. The GXT, power/1-RM testing, and DEXA scan will be repeated the week after completing the training program.

9. Acute biopsy trial

The acute biopsy trial (~ 4 hours) will be performed at least 3 days after completion of the post-testing, and involve performing a single bout of resistance exercise (8 sets of 5 leg press repetitions at 80% 1-RM, 3 min rest between sets). Participants in the HIT+RT and CONT+RT groups will also complete the corresponding form of endurance training 10 minutes before the resistance exercise to mimic training sessions complete during the training program. Upon arrival at the laboratory for the biopsy trial, a plastic cannula will be placed into a forearm vein for blood sampling. After the resistance exercise you will be required to rest quietly in the laboratory for 3 hours. Venous blood samples (~5 mL) will be drawn from the cannula at rest, and after 0, 2, 5, 10, 30, 60, 120 and 180 minutes recovery from resistance exercise. Muscle biopsies will be obtained from an outer thigh muscle (i.e., the vastus lateralis) immediately before, and 1 and 3 hours after exercise (3 biopsies in total) to investigate how the muscle responds to the exercise during early recovery. During this 3-hour recovery period you will be able to complete quiet activities (e.g., read, watch movies, listen to music, etc.).

10. Exercise and diet control

For 24 hours prior to the pre-testingGXT,power/1-RM, DEXA scan and first training session, you will be asked to refrain from any structured exercise and record a detailed food diary. You will then replicate this

dietary intake as accurately as possible for the 24 hours prior to all post-training testing sessions and the acute biopsy trial. On the morning of all testing sessions, the first training session and acute biopsy trial, you will be required to report to the laboratory after an 8-10 h fast. Additionally, you will be required to record another detailed food diary for three consecutive days during the first and last week of training to determine whether your dietary habits change during the training period.

11. Exercise monitoring

During the study, you are allowed to maintain your normal levels of physical activity outside of the study. However, it is important that any additional exercise is recorded for the purposes of measuring how much extra exercise participants do outside of the study. To do this, the student researcher will send you an online training diary questionnaire via email. This training diary will need to be completed 30 minutes after each exercise session performed outside of the study, and submitted via email to the student researcher. The questionairre consists of 4 simple questions regarding the exercise session you have just performed.

What will I gain from participating?

While we cannot guarantee that you will gain any benefits from your participation in this study, you will receive high-quality exercise training supervised by sport scientists in a state-of-the-art research facility. The training is expected to significantly improve various aspects of your health and fitness. In addition, you will also receive potentially valuable information regarding your aerobic fitness and strength levels, and an individualised report on your potential fitness improvements following the training period.

How will the information I give be used?

All data collected will be stored under alphanumeric codes (i.e., without your name or personal details) which will only be identifiable by the researchers. All muscle samples collected will be used to analyse some proteins and genes involved with adaptations to training. The data that will be collected during the study may be used in a thesis, at conference presentations and published in peer-reviewed scientific journals. All data will be presented anonymously so your confidentiality is maintained. With your written consent, photographs or videos may be taken during experimental trials for use in presentations or to assist in future experimental set-ups. Any images will only be taken with your written consent and in all cases you will be de-identified.

What are the potential risks of participating in this project?

The procedures involved in participating in this study are of low risk. Nevertheless, as in any invasive and exercise procedure, there are small risks and some discomfort that may be experienced:

Exercise testing and training

You will experience the fatigue associated with strenuous exercise, particularly during the GXT. Nevertheless, as in any physical activity, there is a very small possibility of injuries that include, but are not restricted to; muscle, ligament or tendon damage, breathing irregularities and dizziness. There is a high probability that you might experience mild muscle soreness for 2-3 days following the 1-RM strength testing, however this will not be more severe than is typically experienced after unaccustomed resistance exercise. There is also a small risk of muscle, ligament or tendon injury during the 1-RM test. However, all protocols are commonly performed in exercise physiology laboratories and potential risks to participants have been minimised by employing appropriate warm-up procedures and researcher supervision.

Intravenous cannulation/venepuncture

Needle insertion into a vein is required for placement of a cannula into a forearm vein and for venepuncture. During the needle insertion you will feel minor to moderate discomfort. However, for the intravenous cannulation (GXT and acute biopsy trial only) the needle is quickly removed and only a flexible plastic tube remains in your vein for the duration of blood sampling (approximately 45 min and 4 hours for the GXT and acute biopsy trial, respectively). When the cannula is removed, direct pressure will be applied to the area to reduce the chances of bruising. Cannulas are routinely placed into veins of participants in clinical research studies and in hospital patients. The risks of IV cannulation are low, but very occasionally significant bruising or infection can occur. The researchers are qualified and experienced in venous cannula placement, venepuncture, and the use of sterile techniques.

Muscle biopsy

The muscle biopsy will be performed by a qualified medical doctor who is experienced in taking muscle biopsies. Xylocaine, a local anaesthetic, will be injected at the site of the muscle biopsy (vastus lateralis – mid outer thigh). The anaesthetic may burn or sting when injected before the area becomes numb. After it becomes numb minimal or no discomfort will be experienced during the procedure. Only slight pressure or a "pulling" sensation will be felt. To extract the muscle biopsy a small incision needs to be made where the muscle biopsy needle will be inserted. After the incision has been made a Bergstrom needle will then be inserted to extract a small muscle sample (approx. 2 rice grains in size). Once the local anaesthetic has worn off and for the next day or two the area will likely feel like you have been "corked". You should not feel any discomfort after 2-3 days. In rare cases haematomas have been reported, although these symptoms typically disappear within a week. On very rare occasions, altered sensation (numbness or tingling) on the skin near the site of the biopsy has been reported; however this sensation disappears over a period of a few weeks to months.

DEXA scan

During the DEXA scan you will be exposed to a very small level of radiation (~0.5 µSv per whole body scan), which is substantially less than a 7 hour plane flight (50 µSv) or a standard chest X-ray (40 µSv). The total dose of radiation you will be exposed to across the two DEXA scans conducted during the study has been assessed by a Medical Physicist and the radiation dose determined to be safe and of negligible risk.

Should you become distressed as a result of your participation in this study please feel free to consult Prof Mark Anderson (registered psychologist at Victoria University) free-of-charge on (03) 99195413 or at mark.andersen@vu.edu.au.

Who is conducting this study?

To express your interest in participating, or further information regarding this research, please contact:

Mr Jackson Fyfe
PhD researcher
Mob: 0419 371 076
Email: jackson.fyfe@live.vu.edu.au

Any queries about your participation in this project may be directed to the Chief Investigator listed above.
If you have any queries or complaints about the way you have been treated, you may contact the Ethics Secretary, Victoria University Human Research Ethics Committee, Office for Research, Victoria University, PO Box 14428, Melbourne, VIC, 8001 or phone (03) 9919 4781.

Appendix C: Cardiovascular risk factor form used for Study 1 and 2 (Chapters 3, 4, and 5)

CARDIOVASCULAR AND OTHER RISK FACTOR QUESTIONNAIRE

In order to be eligible to participate in the experiment investigating: *"The effect of endurance exercise intensity on acute molecular responses to subsequent resistance exercise in recreationally active males* ", you are required to complete the following questionnaire which is designed to assess the risk of you having a cardiovascular event occurring during an exhaustive exercise bout.

Name: _____ Date of birth: _____

Address: _____ Phone: _____

Age: _____ years Weight: _____ kg Height: _____ cm Gender: M F

Give a brief description of your average activity pattern in the past 2 months:

_____

_____

_____

Circle the appropriate response to the following questions.

1.	Are you overweight?	Yes	No	Don't know
2.	Do you smoke?	Yes	No	Social
3.	Are you an asthmatic?	Yes	No	Don't Know
4.	Are you a diabetic?	Yes	No	Don't Know
5.	Does your family have a history of diabetes?			
		Yes	No	Don't Know
6.	Do you have a thyroid disorder?			
		Yes	No	Don't Know
7.	Does your family have a history of thyroid disorders?			

Yes No Don't Know

8. Do you have a pituitary disorder?

Yes No Don't Know

9. Does your family have a history of pituitary disorders?

Yes No Don't Know

10. Do you have a heart rhythm disturbance?

Yes No Don't Know

11. Do you have a high blood cholesterol level?

Yes No Don't Know

12. Do you have elevated blood pressure?

Yes No Don't Know

13. Are you being treated with diuretics?

Yes No Don't Know

14. Are you on any other medications?

Yes No Don't Know

List all medications:

_____

_____

15. Do you think you have any medical complaint or any other reason which you know of which you think may prevent you from participating in strenuous exercise?

Yes No

If Yes, please elaborate

_____

16. Have you had any musculoskeletal problems that have required medical treatment (eg, broken bones, joint reconstruction etc)?

Yes No

If Yes, please provide details (including dates)

_____

17. Does your family have a history of premature cardiovascular problems (e.g. heart attack, stroke)?

Yes No Don't Know

I, _____, believe that the answers to these questions are true and correct.

Signed: _____ Date: _____

Appendix D: Informed consent form used for Study 1 (Chapter 3)

CONSENT FORM FOR PARTICIPANTS INVOLVED IN RESEARCH

INFORMATION TO PARTICIPANTS:

You are invited to participate in research into the effects of endurance exercise intensity on interference of the anabolic response to subsequent resistance exercise.

Aims of project

Simultaneously incorporating both strength and endurance training into a training program is known as concurrent training. While this is a common training approach that combines the benefits of both exercise modes, concurrent training often results in compromised muscle mass, strength and power gains compared to undertaking strength training alone. However, very little is known regarding the factors which worsen or reduce this interference effect and the mechanisms by which this occurs.

Recently, high-intensity interval training (HIT) has become a popular and time-efficient training approach for improving aspects of health and performance. However, very little is currently known regarding the effect of high-intensity endurance exercise on concurrent interference when performed concurrently with resistance exercise.

This project will investigate the effect of endurance exercise intensity (i.e., HIT) on interference of the anabolic responses to subsequent resistance exercise when performed in a concurrent manner.

Procedures involved

- Initially you will attend a familiarisation session (~1 hour) so that you are comfortable with all testing procedures to be undertaken during subsequent sessions.

- You will then undertake a preliminary testing session (~1.5 hours), during which you will undertake a graded exercise test (GXT) to determine your maximal aerobic capacity (VO_{2max}), lactate threshold (LT) and peak power (W_{max}), and a one repetition maximum (1-RM) single leg press test to determine your maximal lower body strength

- Approximately one week later, you will begin the first of three experimental trials (~ 5 hours each) each separated by approximately two weeks. These trials include both moderate or high-intensity cycling and resistance exercise, or resistance exercise only, along with blood and muscle sampling.

The total time commitment for this study is approximately 17.5 hours across five separate visits.

Risks involved

The procedures involved in participating in this study are of low risk. Nevertheless, as in any invasive and exercise procedure, there are small risks and some discomfort that may be experienced.

All potential risks associated with participation in this study are fully explained in the information to participants form.

CERTIFICATION BY SUBJECT

I, …….. (full name)

of ……. (street address)

…….. (suburb)……..(postcode)

Phone: ……

Email: ……

certify that I am at least 18 years old and that I am voluntarily giving my consent to participate in the study: "The effect of endurance exercise intensity on the acute molecular responses induced by subsequent resistance in recreationally active males" exercise being conducted at Victoria University by Mr Jackson Fyfe from the School of Sport and Exercise Science.

I certify that the objectives of the study, together with any risks and safeguards associated with the procedures listed hereunder to be carried out in the research, have been fully explained to me by:

Mr Jackson Fyfe

and that I freely consent to participation involving the below mentioned procedures:

- Graded exercise test (VO_{2max} and LT)
- Maximal strength (1-RM) test
- Moderate/high-intensity cycling and resistance exercise
- Blood and muscle sampling

I certify that I have had the opportunity to have any questions answered and that I understand that I can withdraw from this study at any time and that this withdrawal will not jeopardise me in any way.

ADDITIONAL CONSENT

I also agree to allow photographs or video of me without identifying features to be used in publications or conference presentations. I understand that I am free to withdraw my consent for this at any time without prejudice.

☐ Yes ☐ No

I have been informed that the information I provide will be kept confidential.

Signed: _____

Date: _____

Any queries about your participation in this project may be directed to the student researcher:

Mr Jackson Fyfe
Tel: (03) 9919 4066
Mob: 0419 371 076

Email: jackson.fyfe@live.vu.edu.au

If you have any queries or complaints about the way you have been treated, you may contact the Research Ethics and Biosafety Manager, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 or phone (03) 9919 4148.

Appendix E: Informed consent form used for Study 2 (Chapters 4 and 5)

CONSENT FORM FOR PARTICIPANTS INVOLVED IN RESEARCH

INFORMATION TO PARTICIPANTS:

You are invited to participate in research investigating how different intensities of endurance exercise affect the benefits of resistance training when performed simultaneously.

Aims of project

Simultaneously incorporating both strength and endurance exercise into a training program is known as concurrent training. While this is a common training approach that combines the benefits of both exercise modes, concurrent training often results in less muscle mass and strength improvements compared to if only strength training were performed. Although very little is known about the factors which worsen or lessen this 'interference effect', high endurance exercise intensities and/or volumes have been implicated.

High-intensity interval training (HIT) has recently become a popular and time-efficient training approach for improving multiple aspects of health and performance. However, whether HIT is a favourable exercise strategy to use when trying to simultaneously improve strength and muscle mass (i.e., concurrent training) is unclear. Potentially, higher-intensity endurance exercise may interfere with the ability to perform, and respond to, subsequent resistance exercise.

This project will compare the effects of HIT versus lower-intensity, traditional continuous endurance exercise on the interference effect when performed concurrently with resistance exercise, compared to undertaking resistance exercise alone.

Procedures involved

- The first visit will be a familiarisation session (~1 hour) during which you will be asked to complete some questionnaires regarding your health, medical and exercise history. You will then be familiarised with all testing procedures to be undertaken during subsequent sessions.

- You will then undertake preliminary testing across the next two sessions to evaluate various aspects of your fitness. The first session (~1 hour) involves a graded exercise test (GXT) performed on a cycle ergometer to determine your aerobic fitness, and a DEXA scan to estimate your body composition. The second session (~1 hour) will involve 1-repetition maximum (1-RM) strength and power (jump) testing.

- The following week you will begin the 8-week training program. Training sessions will be performed 3 times per week and consist of either high-intensity (HIT) cycling followed by resistance exercise continuous (CONT) cycling followed by resistance exercise, or resistance exercise only, depending on the group you are randomly allocated to. Each training session will last between ~40 – 110 minutes. Before the first training session only, a single resting muscle biopsy will obtained from an outer thigh muscle.

- At least 3 days after completing the training program, you will undergo an acute biopsy trial (~ 4 hours), whereby you will perform a single bout of resistance exercise. Muscle biopsies will be

obtained immediately before, and 1 and 3 hours after the resistance exercise (3 biopsies in total) to investigate how the muscle responds to the exercise during early recovery. Blood samples will also be obtained from a cannula inserted into a forearm vein at regular intervals throughout the trial.

- After completion of the training program, you will repeat all preliminary testing to determine how you have responded to the training program. Strength and jump testing will be performed as part of the final training session, and the GXT and DEXA scan will be repeated at least 3 days after the acute biopsy trial.

Risks involved

The procedures involved in participating in this study are of low risk. Nevertheless, as in any invasive and exercise procedure, there are small risks and some discomfort that may be experienced.

All potential risks associated with participation in this study are fully explained in the 'Information to Participants' form.

CERTIFICATION BY SUBJECT

I, …….. (full name)

of …… (street address)

…….. (suburb)……..(postcode)

Phone: ……

Email: ……

certify that I am at least 18 years old and that I am voluntarily giving my consent to participate in the study: "The effect of endurance training intensity on interference between concurrent resistance and endurance exercise in recreationally-active males" being conducted at Victoria University by Mr Jackson Fyfe from the College of Sport and Exercise Science.

I certify that the objectives of the study, together with any risks and safeguards associated with the procedures listed hereunder to be carried out in the research, have been fully explained to me by:

Mr Jackson Fyfe

and that I freely consent to participation involving the below mentioned procedures:

- Graded exercise test (GXT)
- Maximal strength (1-RM) and power (jump) testing
- DEXA scan
- 8-week training program conducted three (3) times per week
- Blood and muscle sampling

I certify that I have had the opportunity to have any questions answered and that I understand that I can withdraw from this study at any time and that this withdrawal will not jeopardise me in any way.

ADDITIONAL CONSENT

I also agree to allow photographs or video of me without identifying features to be used in publications or conference presentations. I understand that I am free to withdraw my consent for this at any time without prejudice.

☐ Yes ☐ No

CONSENT FOR ADDITIONAL ANALYSES

By ticking this box, I agree to have my blood and cell samples preserved and analysed for molecular markers related to immune cell function, based on the findings of this study.

☐ Yes ☐ No

I have been informed that the information I provide will be kept confidential.

Signed: _____

Date: _____

Any queries about your participation in this project may be directed to the student researcher:

Mr Jackson Fyfe
Mob: 0419 371 076
Email: jackson.fyfe@live.vu.edu.au

If you have any queries or complaints about the way you have been treated, you may contact the Research Ethics and Biosafety Manager, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 or phone (03) 9919 4148.

Appendix F: Muscle biopsy and venous catheterisation questionnaire form used for Study 1 and 2 (Chapters 3, 4, and 5)

MUSCLE BIOPSY & VENOUS CATHETERISATION QUESTIONNAIRE

"The effect of endurance exercise intensity on acute molecular responses to subsequent resistance exercise in recreationally active males."

NAME: _____

ADDRESS: _____

_____

DATE: _____ AGE: _____ years

1. Have you or your family suffered from any tendency to bleed excessively? (e.g. Haemophilia) or bruise very easily? Yes No Don't Know
   If yes, please elaborate
_____

2. Are you allergic to local anaesthetic? Yes No Don't Know
   If yes, please elaborate
_____

3. Do you have any skin allergies? Yes No Don't Know
   If yes, please elaborate
_____

4. Have you any other allergies? Yes No Don't Know
   If yes, please elaborate
_____

5. Are you currently on any medication? Yes No
   If yes, what is the medication?
_____

6. Do you have any other medical problems? Yes No
   If yes, please elaborate
_____

7. Have you ever fainted when you had an injection or blood sample taken?

Yes No Don't know

If yes, please elaborate

_____

8. Have you previously had heparin infused or injected?

Yes No Don't know

If yes, please elaborate

_____

9. Do you or other members of your family have Raynauds disease, or suffer from very poor circulation in the fingers, leading to painful fingers that turn white/blue?

Yes No Don't know

If yes, please elaborate

_____

To the best of my knowledge, the above questionnaire has been completely accurately and truthfully.

Signature: _____ Date: _____

Appendix G: 24-hour food diary form used for Study 2 (Chapters 4 and 5)

INSTRUCTIONS FOR THE 24 HOURS BEFORE PHYSIOLOGICAL TESTING

For the research project entitled: "*The effect of endurance exercise intensity on interference between concurrent endurance and resistance exercise in recreationally-active males*".

Please read carefully the following instructions to be followed prior to the following testing sessions:

1. Graded exercise test (GXT)
2. Maximal strength (1-RM) and power (CMJ) testing
3. Body composition testing (DEXA)
4. First training session

The reasons for the following restrictions are to ensure, as best as possible, that each participant reports to the laboratory in a similar physiological state when testing is repeated after the training intervention.

Please avoid the following for the 24 hours prior to all testing sessions:

- Structured or strenuous exercise of any kind
- Caffeine or alcohol consumption

Please do the following for 24 hours before all testing sessions:

- Record all food and drink that is consumed during the entire 24 hour period (as accurately as possible)
- Be as specific as possible: include brands of foods (e.g., Helga's bread), amounts (e.g., 2 slices, 20 grams, estimate where possible), and types (e.g., mixed grain).

Please do the following on the morning of all testing sessions:

- On the morning of all testing sessions, you will be required to report to the lab after an overnight fast (before you have had breakfast). Please do not eat or drink anything (apart from water) in the morning before arriving at the lab.

The 24-hour food diary you complete before all testing sessions will then be returned to you before you repeat the particular testing session after the training intervention. This will allow you to repeat the food diary as accurately as possible.

If you have any questions whatsoever, please do not hesitate to contact me either by phone or email:

Jackson Fyfe
Mobile: 0419 371 076
Email: jackson.fyfe@live.vu.edu.au

24-HOUR FOOD DIARY RECORD

For the research project: "*The effect of endurance exercise intensity on interference between concurrent endurance and resistance exercise in recreationally-active males*".

Name: _____

Date of food diary: ____/_____/_____ Test: _____

Date of testing: ____/_____/_____ Arrival time: _____

Please record all food and drink consumed for the entire 24-hour period before fitness testing.

Meal	Food (include brand, type, flavour etc.)	Amount (estimate in grams or litres if possible)
Breakfast		
Mid-morning snack		
Lunch		
Mid-afternoon snack		
Dinner		
Dessert/snack		
Approx. water intake:	mL	

Appendix H: 72-hour food diary form used for Study 2 (Chapters 4 and 5)

INSTRUCTIONS FOR THE 72-HOUR

72-HOUR FOOD DIARY RECORD

For the research project: "*The effect of endurance exercise intensity on interference between concurrent endurance and resistance exercise in recreationally-active males*".

Name: _____

Date of food diary: ____/_____/_____ to: ____/_____/_____

Pre- or post-training: _____

DAY ONE: ____/_____/_____

Meal	Food (include brand, type, flavour etc.)	Amount (estimate in grams or litres if possible)
Breakfast		
Mid-morning snack		
Lunch		
Mid-afternoon snack		
Dinner		

Dessert/snack		
Approx. water intake:	mL	

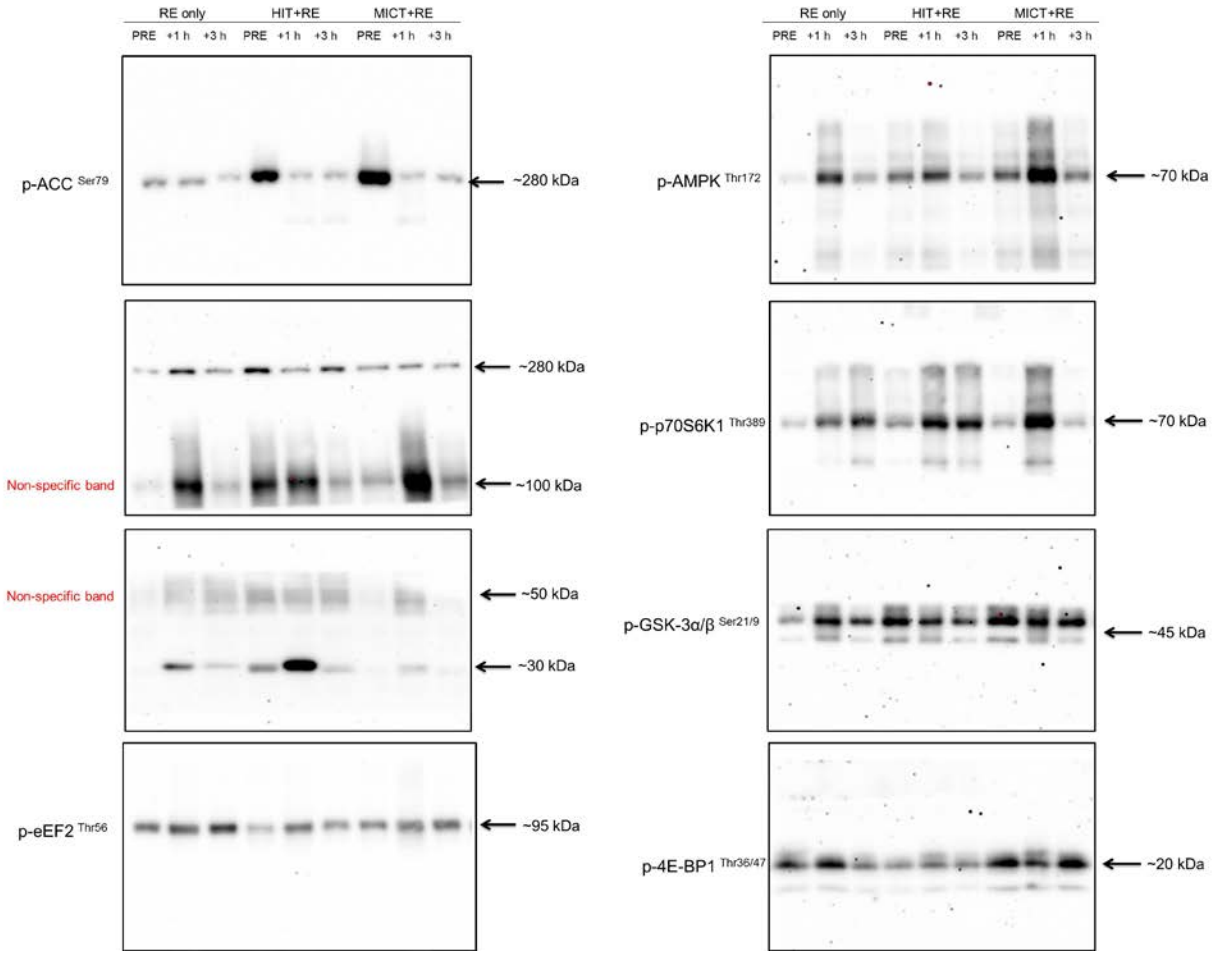
DAY TWO: ____/_____/_____

Meal	Food (include brand, type, flavour etc.)	Amount (estimate in grams or litres if possible)
Breakfast		
Mid-morning snack		
Lunch		
Mid-afternoon snack		
Dinner		
Dessert/snack		
Approx. water intake:	mL	

DAY THREE: ____/_____/_____

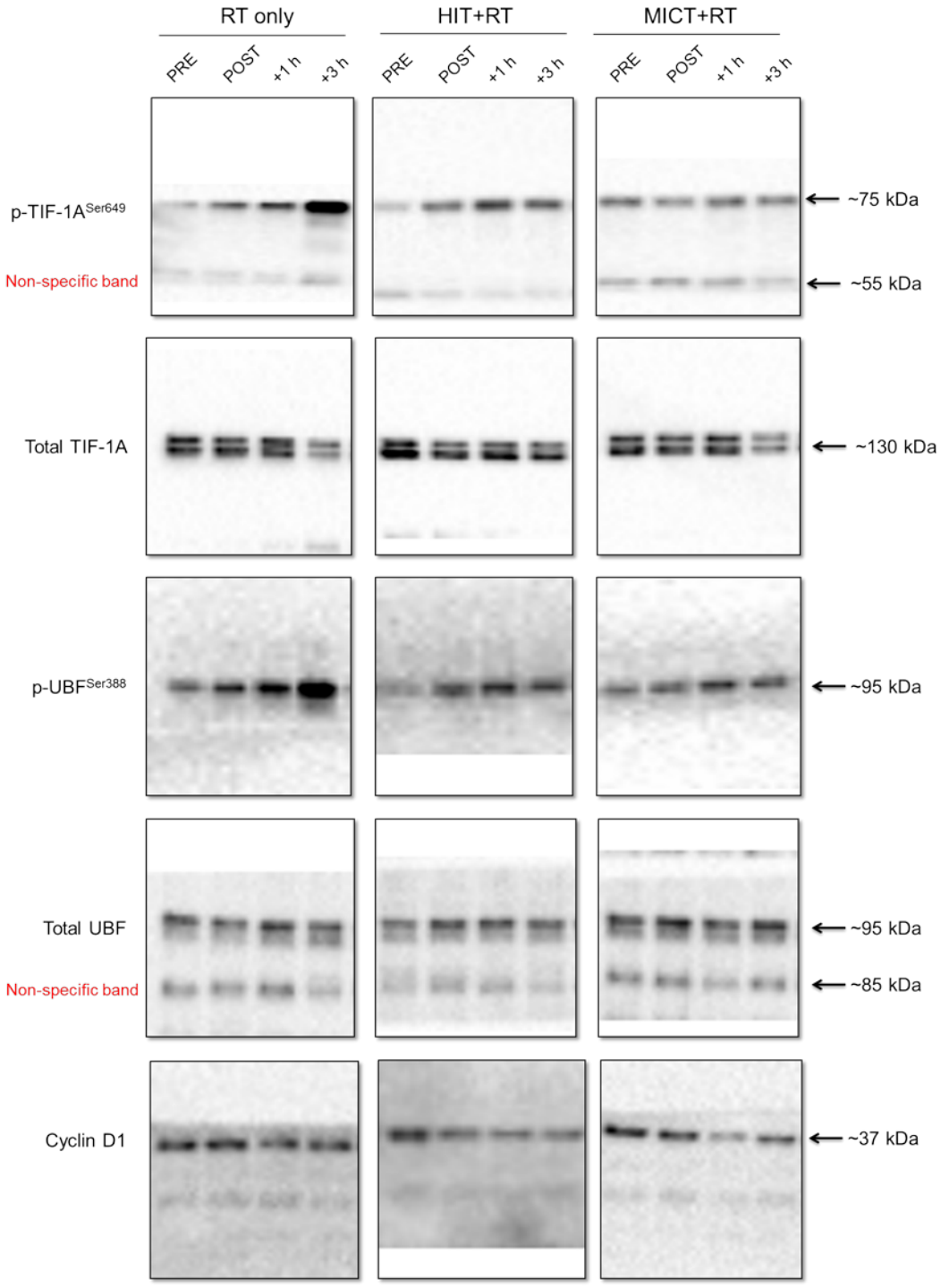
Meal	Food (include brand, type, flavour etc.)	Amount (estimate in grams or litres if possible)
Breakfast		
Mid-morning snack		
Lunch		
Mid-afternoon snack		
Dinner		
Dessert/snack		
Approx. water intake:	mL	

Appendix I: Extended representative western blot images for Study 1 (Chapter 3)[7]

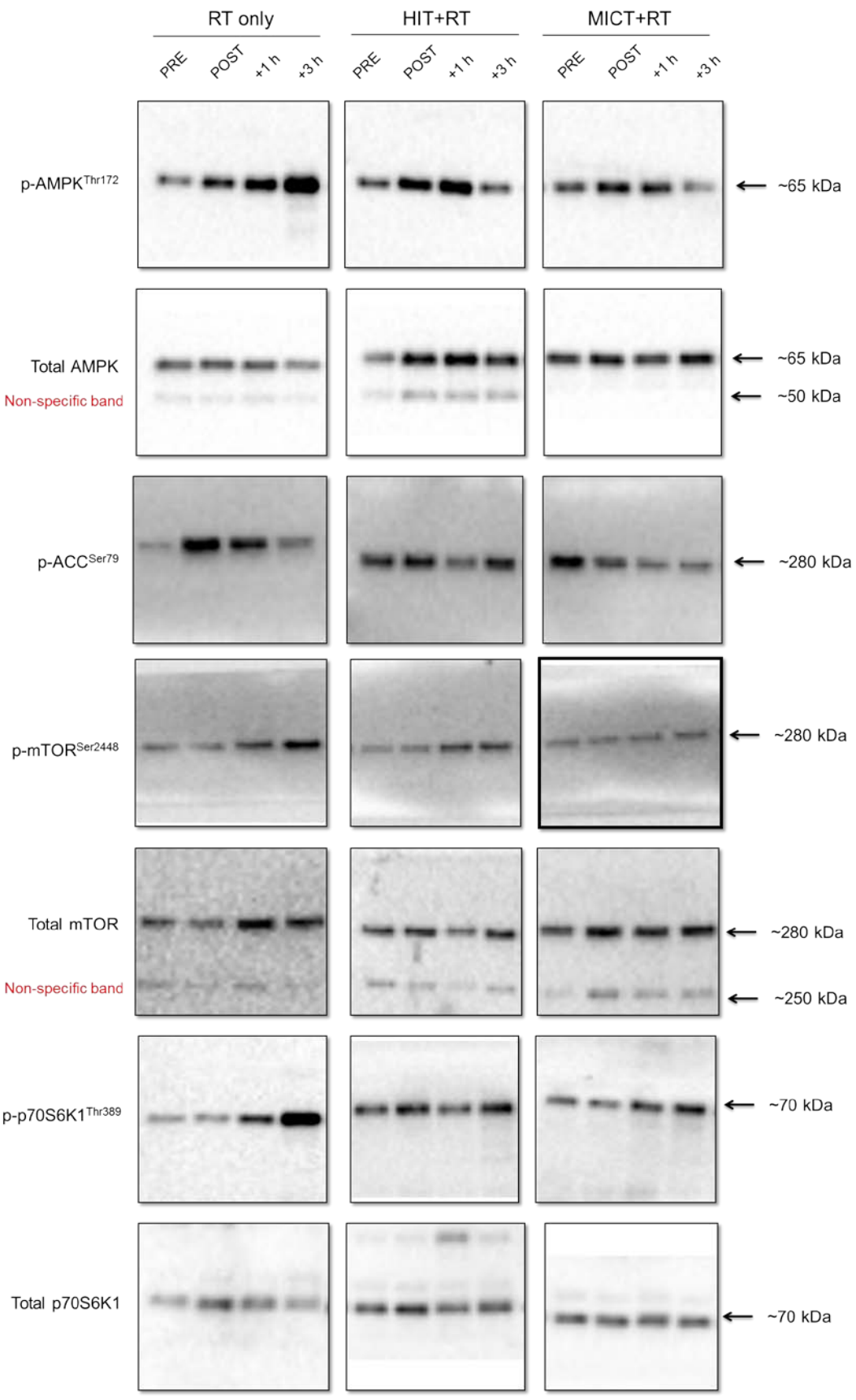


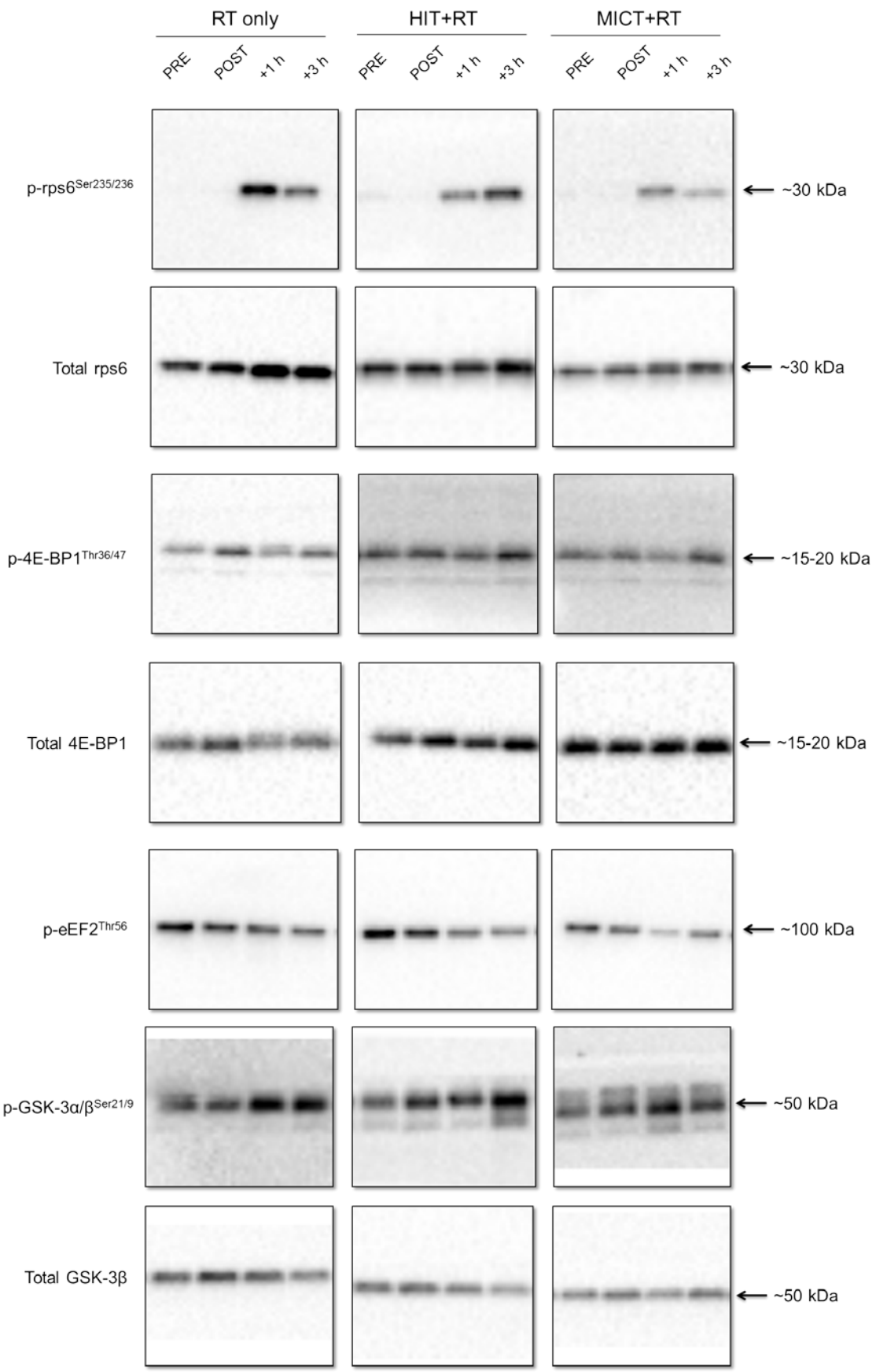
[7] After transfer, membranes were cut to approximately the size displayed in the images above to allow for probing with multiple primary antibodies on a single membrane/gel. The above images therefore display the largest available membrane area probed with each respective primary antibody.

Appendix J: Extended representative western blot images for Study 2 (Chapter 5)[8]



[8] After transfer, membranes were cut to approximately the size displayed in the images above to allow for probing with multiple primary antibodies on a single membrane/gel. The above images therefore display the largest available membrane area probed with each respective primary antibody. Images for each protein and training group were obtained from separate gels and are therefore separated to indicate this.


RT only
HIT+RT
MICT+RT
PRE
POST
+1 h
+3 h
p-AMPK Thr172
~65 kDa
Total AMPK
Non-specific band
~65 kDa
~50 kDa
p-ACC Ser79
~280 kDa
p-mTOR Ser2448
~280 kDa
Total mTOR
Non-specific band
~280 kDa
~250 kDa
p-p70S6K1 Thr389
~70 kDa
Total p70S6K1
~70 kDa


RT only
HIT+RT
MICT+RT
PRE
POST
+1 h
+3 h
p-rps6Ser235/236
~30 kDa
Total rps6
~30 kDa
p-4E-BP1Thr36/47
~15-20 kDa
Total 4E-BP1
~15-20 kDa
p-eEF2Thr56
~100 kDa
p-GSK-3α/βSer21/9
~50 kDa
Total GSK-3β
~50 kDa

Appendix K: Raw data for Study 1 (Chapter 3)

Study 1: Participant characteristics

Participant	Height (cm)	Mass (kg)	1-RM leg press (left leg [kg])	1-RM leg press (right leg [kg])	$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Lactate threshold (W)	Peak aerobic power (W)
1	175.7	75.7	178	183	40.7	134	215
2	166.0	67.5	168	173	43.3	176	246
3	179.7	73.3	110.5	110.5	64.2	221	327
4	177.5	110.0	228	223	34	151	254
5	186.9	79.2	178	178	51.8	212	321
6	178.9	80.1	138	153	41.8	150	225
7	183.4	89.3	178	203	44.4	180	285
8	178.6	94.6	210.5	188	45.3	208	309
Mean	**178.3**	**83.7**	**174**	**176**	**45.7**	**179**	**273**
SD	**6.1**	**13.7**	**37**	**34**	**9.0**	**32**	**44**

Study 1: Muscle glycogen data (mmol·kg^{-1} dry mass)

Trial	Participant	PRE	+1 h	+3 h
RE	1	479.6	493.6	446.7
RE	2	510.1	546.4	474.8
RE	3	466.5	422.4	490.6
RE	4	305.5	539.9	440.8
RE	5	294.1	362.2	265.1
RE	6	462.3	524.1	496.7
RE	7	463.2	387.8	396.0
RE	8	382.2	405.4	398.7
	Mean	**420.4**	**460.2**	**426.2**
	SD	**82.7**	**73.9**	**75.3**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	62.1	62.3	183.9
HIT+RE	2	176.4	203.0	238.5
HIT+RE	3	365.7	112.0	464.2
HIT+RE	4	300.6	258.0	264.2
HIT+RE	5	225.8	183.6	108.4
HIT+RE	6	299.7	172.8	229.7
HIT+RE	7	324.8	231.1	243.2
HIT+RE	8	117.7	306.5	257.4
	Mean	**234.1**	**191.1**	**248.7**
	SD	**107.4**	**78.2**	**100.8**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	203.7	214.6	367.0
MICT+RE	2	222.3	178.8	254.5
MICT+RE	3	207.8	115.1	159.0
MICT+RE	4	308.4	351.2	251.9
MICT+RE	5	179.3	233.2	229.0
MICT+RE	6	248.3	320.9	355.3
MICT+RE	7	326.7	434.5	491.5
MICT+RE	8	348.7	388.0	320.3
	Mean	**255.7**	**279.5**	**303.6**
	SD	**63.8**	**111.0**	**102.7**

Study 1: Western blotting data

p-mTOR Ser 2448
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	1.10	1.95	1.68
RE	2	0.22	0.51	0.50
RE	3	0.44	1.67	0.80
RE	4	2.01	1.56	1.06
RE	5	0.82	0.93	0.43
RE	6	1.83	1.34	1.04
RE	7	1.13	1.09	1.98
RE	8	0.46	0.58	0.08
	Mean	**1.00**	**1.20**	**0.95**
	SD	**0.65**	**0.52**	**0.64**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	1.62	1.31	0.51
HIT+RE	2	0.98	1.15	0.77
HIT+RE	3	2.08	0.82	1.40
HIT+RE	4	1.66	1.12	1.59
HIT+RE	5	1.83	1.54	1.15
HIT+RE	6	2.41	2.30	1.75
HIT+RE	7	1.93	3.53	3.48
HIT+RE	8	1.11	1.86	1.52
	Mean	**1.70**	**1.70**	**1.52**
	SD	**0.48**	**0.87**	**0.90**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	1.19	0.46	1.03
MICT+RE	2	0.82	0.33	1.37
MICT+RE	3	0.84	0.83	0.66
MICT+RE	4	0.67	0.65	0.05
MICT+RE	5	0.67	0.44	0.07
MICT+RE	6	1.08	1.89	2.29
MICT+RE	7	2.93	1.30	1.79
MICT+RE	8	0.44	0.49	0.63
	Mean	**1.08**	**0.80**	**0.99**
	SD	**0.78**	**0.54**	**0.80**

p-p70S6K1 Thr389
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	1.99	3.73	2.32
RE	2	0.69	1.73	0.83
RE	3	0.41	4.20	0.93
RE	4	0.80	2.18	0.63
RE	5	2.01	2.62	2.40
RE	6	0.36	0.76	0.79
RE	7	0.99	2.77	2.94
RE	8	0.73	2.12	2.11
	Mean	**1.00**	**2.51**	**1.62**
	SD	**0.65**	**1.09**	**0.91**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	0.93	2.04	1.05
HIT+RE	2	1.29	1.51	0.89
HIT+RE	3	3.30	3.38	0.94
HIT+RE	4	1.73	1.88	2.25
HIT+RE	5	0.99	1.26	2.04
HIT+RE	6	1.14	4.07	1.72
HIT+RE	7	1.00	3.46	3.27
HIT+RE	8	2.95	1.98	2.27
	Mean	**1.67**	**2.45**	**1.80**
	SD	**0.94**	**1.04**	**0.83**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	2.46	1.90	2.64
MICT+RE	2	1.37	2.69	1.00
MICT+RE	3	1.03	4.49	1.43
MICT+RE	4	1.13	2.16	0.72
MICT+RE	5	1.08	1.08	1.58
MICT+RE	6	3.66	1.35	2.58
MICT+RE	7	1.05	3.72	0.80
MICT+RE	8	0.63	3.05	0.77
	Mean	**1.55**	**2.55**	**1.44**
	SD	**1.01**	**1.17**	**0.79**

p-4E-BP1 Thr37/46
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	0.49	0.39	0.42
RE	2	0.29	0.25	0.33
RE	3	0.16	0.15	0.21
RE	4	0.15	0.20	0.52
RE	5	1.73	3.00	0.59
RE	6	2.88	2.86	1.24
RE	7	1.30	1.43	0.69
RE	8	1.00	1.18	0.57
	Mean	**1.00**	**1.18**	**0.57**
	SD	**0.95**	**1.18**	**0.31**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	0.24	0.21	0.28
HIT+RE	2	0.39	0.43	0.42
HIT+RE	3	0.20	0.27	0.36
HIT+RE	4	0.48	0.41	0.22
HIT+RE	5	1.29	2.57	1.65
HIT+RE	6	1.13	1.40	2.53
HIT+RE	7	0.55	0.61	0.54
HIT+RE	8	0.61	0.84	0.86
	Mean	**0.61**	**0.84**	**0.86**
	SD	**0.40**	**0.80**	**0.82**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	0.20	0.32	0.32
MICT+RE	2	0.43	0.25	0.24
MICT+RE	3	0.41	0.22	0.36
MICT+RE	4	0.42	0.65	0.38
MICT+RE	5	1.46	3.09	2.68
MICT+RE	6	1.28	1.75	1.81
MICT+RE	7	1.19	0.83	1.31
MICT+RE	8	0.77	1.02	1.01
	Mean	**0.77**	**1.02**	**1.01**
	SD	**0.48**	**0.98**	**0.88**

p-eEF2 Thr56
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	0.89	1.20	1.39
RE	2	0.71	0.99	1.03
RE	3	0.97	0.81	1.15
RE	4	1.29	1.18	1.09
RE	5	0.76	0.65	0.42
RE	6	1.04	1.02	1.16
RE	7	1.07	0.99	0.74
RE	8	1.26	0.49	0.21
	Mean	**1.00**	**0.92**	**0.90**
	SD	**0.21**	**0.25**	**0.41**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	1.50	0.97	0.59
HIT+RE	2	0.32	0.74	0.44
HIT+RE	3	1.24	0.60	0.84
HIT+RE	4	1.07	1.13	1.13
HIT+RE	5	0.36	0.70	0.44
HIT+RE	6	0.21	0.63	0.71
HIT+RE	7	0.63	0.77	0.78
HIT+RE	8	0.97	0.56	0.91
	Mean	**0.79**	**0.76**	**0.73**
	SD	**0.47**	**0.20**	**0.24**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	0.49	0.47	0.85
MICT+RE	2	0.55	0.71	0.78
MICT+RE	3	0.50	0.37	0.53
MICT+RE	4	1.14	1.27	0.58
MICT+RE	5	0.25	0.61	0.42
MICT+RE	6	1.58	0.68	0.91
MICT+RE	7	0.78	1.14	0.78
MICT+RE	8	0.48	0.71	0.77
	Mean	**0.72**	**0.75**	**0.70**
	SD	**0.44**	**0.31**	**0.17**

p-rps6 Ser235/236
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	3.49	1.77	2.05
RE	2	0.48	0.93	2.54
RE	3	0.20	2.10	0.56
RE	4	0.60	4.83	1.28
RE	5	0.86	2.76	0.83
RE	6	1.07	0.99	12.01
RE	7	0.91	2.39	7.13
RE	8	0.40	5.76	1.72
	Mean	**1.00**	**2.69**	**3.52**
	SD	**1.05**	**1.74**	**4.01**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	2.12	1.44	1.12
HIT+RE	2	1.78	0.68	0.84
HIT+RE	3	0.91	0.91	0.60
HIT+RE	4	0.65	0.95	2.83
HIT+RE	5	3.22	2.31	13.27
HIT+RE	6	2.10	9.53	3.55
HIT+RE	7	1.70	10.40	14.22
HIT+RE	8	4.33	6.52	2.07
	Mean	**2.10**	**4.09**	**4.81**
	SD	**1.20**	**4.09**	**5.61**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	1.39	0.85	1.08
MICT+RE	2	0.78	0.64	0.63
MICT+RE	3	0.73	0.45	4.18
MICT+RE	4	0.26	2.12	0.40
MICT+RE	5	3.98	1.71	1.94
MICT+RE	6	2.50	9.62	11.09
MICT+RE	7	2.11	8.02	2.79
MICT+RE	8	0.52	1.44	0.59
	Mean	**1.53**	**3.11**	**2.84**
	SD	**1.26**	**3.60**	**3.58**

p-GSK-3β Ser9
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	3.30	5.40	2.77
RE	2	0.21	0.42	0.28
RE	3	1.06	4.76	1.47
RE	4	0.41	0.62	0.93
RE	5	1.65	4.83	1.63
RE	6	0.92	0.95	0.56
RE	7	0.27	1.07	1.05
RE	8	0.17	0.52	0.88
	Mean	**1.00**	**2.32**	**1.20**
	SD	**1.06**	**2.23**	**0.77**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	1.73	2.31	0.76
HIT+RE	2	0.39	0.38	0.35
HIT+RE	3	5.23	4.20	1.82
HIT+RE	4	0.99	0.41	0.48
HIT+RE	5	0.98	5.18	5.06
HIT+RE	6	1.19	2.32	1.27
HIT+RE	7	0.30	1.22	1.11
HIT+RE	8	1.45	0.83	0.85
	Mean	**1.53**	**2.11**	**1.46**
	SD	**1.57**	**1.78**	**1.52**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	2.18	0.94	2.11
MICT+RE	2	0.41	0.45	0.41
MICT+RE	3	4.69	4.47	2.59
MICT+RE	4	0.22	0.90	0.52
MICT+RE	5	4.25	1.10	1.37
MICT+RE	6	3.31	1.14	1.40
MICT+RE	7	0.54	1.22	0.38
MICT+RE	8	0.16	0.80	0.21
	Mean	**1.97**	**1.38**	**1.12**
	SD	**1.90**	**1.27**	**0.89**

p-ACC Ser79
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	0.57	0.58	0.98
RE	2	0.24	0.16	0.37
RE	3	1.42	0.59	0.37
RE	4	2.04	0.48	0.08
RE	5	0.43	0.10	0.01
RE	6	0.89	0.46	0.29
RE	7	0.97	0.87	0.46
RE	8	1.43	0.12	0.01
	Mean	**1.00**	**0.42**	**0.32**
	SD	**0.60**	**0.27**	**0.32**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	6.74	1.73	0.36
HIT+RE	2	4.72	1.52	1.11
HIT+RE	3	9.43	0.67	1.30
HIT+RE	4	3.60	0.69	0.59
HIT+RE	5	4.58	0.78	0.61
HIT+RE	6	4.00	0.69	0.73
HIT+RE	7	5.55	0.55	0.70
HIT+RE	8	4.74	0.31	2.14
	Mean	**5.42**	**0.87**	**0.94**
	SD	**1.88**	**0.49**	**0.57**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	3.88	0.11	0.10
MICT+RE	2	3.28	0.29	0.27
MICT+RE	3	9.83	0.15	0.51
MICT+RE	4	1.25	0.18	0.07
MICT+RE	5	4.70	0.56	0.42
MICT+RE	6	6.06	0.30	0.52
MICT+RE	7	8.74	0.56	0.64
MICT+RE	8	4.63	0.70	0.65
	Mean	**5.29**	**0.35**	**0.40**
	SD	**2.84**	**0.22**	**0.23**

p-AMPK Thr172
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	2.24	3.28	4.52
RE	2	0.41	4.57	1.22
RE	3	0.44	1.31	0.72
RE	4	0.73	3.14	6.80
RE	5	1.95	1.80	3.98
RE	6	0.40	0.35	0.66
RE	7	1.10	5.47	4.53
RE	8	0.74	0.87	3.82
	Mean	**1.00**	**2.60**	**3.28**
	SD	**0.72**	**1.82**	**2.20**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	5.25	3.99	1.00
HIT+RE	2	2.41	4.79	1.60
HIT+RE	3	5.65	5.68	1.50
HIT+RE	4	1.18	1.93	1.45
HIT+RE	5	0.40	0.62	5.96
HIT+RE	6	0.56	1.34	0.55
HIT+RE	7	1.13	8.52	6.48
HIT+RE	8	2.87	2.84	2.89
	Mean	**2.43**	**3.71**	**2.68**
	SD	**2.05**	**2.60**	**2.29**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	0.93	2.12	1.36
MICT+RE	2	2.81	9.32	3.53
MICT+RE	3	1.14	5.12	2.25
MICT+RE	4	1.96	2.53	1.89
MICT+RE	5	11.23	2.74	3.52
MICT+RE	6	2.23	0.87	2.57
MICT+RE	7	1.33	4.97	0.87
MICT+RE	8	0.49	2.54	1.24
	Mean	**2.76**	**3.78**	**2.15**
	SD	**3.50**	**2.65**	**1.01**

Study 1: qPCR mRNA data

MuRF-1 mRNA
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+3 h
RE	1	1.00	0.01
RE	2	2.34	1.71
RE	3	0.67	3.43
RE	4	1.18	3.14
RE	5	0.85	3.28
RE	6	0.60	0.84
RE	7	0.29	2.40
RE	8	1.07	6.18
	Mean	**1.00**	**2.62**
	SD	**0.61**	**1.88**

Trial	Participant	POST	+3 h
HIT+RE	1	0.03	0.03
HIT+RE	2	0.05	0.01
HIT+RE	3	1.12	2.95
HIT+RE	4	0.00	0.10
HIT+RE	5	1.24	39.90
HIT+RE	6	1.07	24.91
HIT+RE	7	0.93	20.49
HIT+RE	8	0.02	0.43
	Mean	**0.56**	**11.10**
	SD	**0.57**	**15.38**

Trial	Participant	POST	+3 h
MICT+RE	1	0.33	0.15
MICT+RE	2	0.00	6.58
MICT+RE	3	3.91	9.78
MICT+RE	4	0.73	4.44
MICT+RE	5	1.55	17.98
MICT+RE	6	0.01	10.75
MICT+RE	7	0.04	5.03
MICT+RE	8	1.37	9.70
	Mean	**0.99**	**8.05**
	SD	**1.33**	**5.33**

Atrogin-1 mRNA
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+3 h
RE	1	0.01	0.02
RE	2	1.48	1.47
RE	3	0.34	0.59
RE	4	2.32	0.55
RE	5	2.39	0.82
RE	6	0.46	0.15
RE	7	0.37	0.33
RE	8	0.63	0.51
	Mean	**1.00**	**0.55**
	SD	**0.94**	**0.45**

Trial	Participant	POST	+3 h
HIT+RE	1	0.01	0.00
HIT+RE	2	0.02	0.00
HIT+RE	3	1.57	2.08
HIT+RE	4	0.18	0.15
HIT+RE	5	0.45	2.07
HIT+RE	6	0.72	1.60
HIT+RE	7	1.00	0.40
HIT+RE	8	0.10	0.04
	Mean	**0.51**	**0.79**
	SD	**0.56**	**0.95**

Trial	Participant	POST	+3 h
MICT+RE	1	0.21	0.00
MICT+RE	2	0.05	0.17
MICT+RE	3	2.05	0.71
MICT+RE	4	0.27	0.14
MICT+RE	5	3.55	4.25
MICT+RE	6	0.15	0.47
MICT+RE	7	0.01	0.90
MICT+RE	8	1.22	0.89
	Mean	**0.94**	**0.94**
	SD	**1.27**	**1.38**

PGC-1α mRNA
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+3 h
RE	1	0.01	0.09
RE	2	2.39	0.82
RE	3	0.29	1.31
RE	4	0.90	1.58
RE	5	0.83	0.90
RE	6	1.41	1.13
RE	7	0.59	2.16
RE	8	1.58	2.89
	Mean	**1.00**	**1.36**
	SD	**0.77**	**0.86**

Trial	Participant	POST	+3 h
HIT+RE	1	0.10	0.06
HIT+RE	2	0.11	0.02
HIT+RE	3	0.39	4.68
HIT+RE	4	0.13	0.10
HIT+RE	5	1.93	28.94
HIT+RE	6	0.75	19.23
HIT+RE	7	0.69	19.47
HIT+RE	8	0.11	0.56
	Mean	**0.53**	**9.13**
	SD	**0.63**	**11.60**

Trial	Participant	POST	+3 h
MICT+RE	1	0.21	0.06
MICT+RE	2	0.08	0.19
MICT+RE	3	1.21	6.53
MICT+RE	4	0.21	1.90
MICT+RE	5	2.01	14.57
MICT+RE	6	0.14	10.01
MICT+RE	7	0.03	17.11
MICT+RE	8	4.89	28.94
	Mean	**1.10**	**9.91**
	SD	**1.69**	**10.02**

Rheb mRNA
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+3 h
RE	1	0.13	1.45
RE	2	1.35	5.81
RE	3	0.31	19.81
RE	4	2.14	8.22
RE	5	2.32	19.50
RE	6	0.55	3.52
RE	7	0.25	9.49
RE	8	0.94	7.20
	Mean	**1.00**	**9.37**
	SD	**0.86**	**6.84**

Trial	Participant	POST	+3 h
HIT+RE	1	0.13	3.02
HIT+RE	2	0.15	1.47
HIT+RE	3	1.49	5.46
HIT+RE	4	1.72	0.00
HIT+RE	5	1.58	12.72
HIT+RE	6	1.30	7.83
HIT+RE	7	0.09	10.61
HIT+RE	8	0.06	2.34
	Mean	**0.81**	**5.43**
	SD	**0.77**	**4.57**

Trial	Participant	POST	+3 h
MICT+RE	1	0.15	1.57
MICT+RE	2	0.11	4.46
MICT+RE	3	5.48	18.90
MICT+RE	4	0.30	4.92
MICT+RE	5	2.39	10.70
MICT+RE	6	0.00	12.02
MICT+RE	7	0.16	12.91
MICT+RE	8	1.05	19.55
	Mean	**1.21**	**10.63**
	SD	**1.91**	**6.63**

TSC2 mRNA
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+3 h
RE	1.00	0.24	0.09
RE	2.00	2.28	1.53
RE	3.00	1.01	0.61
RE	4.00	0.12	1.23
RE	5.00	0.54	2.30
RE	6.00	1.25	0.42
RE	7.00	0.78	2.57
RE	8.00	1.79	1.05
	Mean	**1.00**	**1.23**
	SD	**0.81**	**0.95**

Trial	Participant	POST	+3 h
HIT+RE	1.00	0.03	1.84
HIT+RE	2.00	1.63	0.14
HIT+RE	3.00	1.18	1.16
HIT+RE	4.00	1.48	1.64
HIT+RE	5.00	4.11	0.86
HIT+RE	6.00	2.11	3.22
HIT+RE	7.00	0.70	3.33
HIT+RE	8.00	0.16	1.74
	Mean	**1.43**	**1.74**
	SD	**1.71**	**1.45**

Trial	Participant	POST	+3 h
MICT+RE	1.00	0.91	0.49
MICT+RE	2.00	0.11	2.11
MICT+RE	3.00	1.14	3.75
MICT+RE	4.00	0.96	2.21
MICT+RE	5.00	1.08	0.32
MICT+RE	6.00	1.02	4.12
MICT+RE	7.00	0.06	2.46
MICT+RE	8.00	3.12	2.20
	Mean	**1.05**	**2.21**
	SD	**1.43**	**1.45**

Myostatin mRNA
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+3 h
RE	1	0.04	0.26
RE	2	1.05	1.20
RE	3	0.83	1.88
RE	4	0.92	1.32
RE	5	1.48	2.36
RE	6	1.83	0.76
RE	7	1.66	3.55
RE	8	0.19	0.26
	Mean	**1.00**	**1.45**
	SD	**0.65**	**1.12**

Trial	Participant	POST	+3 h
HIT+RE	1	0.24	1.03
HIT+RE	2	1.33	1.39
HIT+RE	3	3.93	0.62
HIT+RE	4	1.45	1.56
HIT+RE	5	2.84	5.90
HIT+RE	6	1.14	0.64
HIT+RE	7	1.26	2.20
HIT+RE	8	0.41	0.34
	Mean	**1.58**	**1.71**
	SD	**1.23**	**1.80**

Trial	Participant	POST	+3 h
MICT+RE	1	0.46	0.21
MICT+RE	2	0.56	1.17
MICT+RE	3	0.56	2.78
MICT+RE	4	0.56	1.27
MICT+RE	5	0.81	1.01
MICT+RE	6	0.59	1.19
MICT+RE	7	0.33	3.06
MICT+RE	8	0.59	0.39
	Mean	**0.56**	**1.38**
	SD	**0.14**	**1.02**

Fox-O1 mRNA
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+3 h
RE	1	1.83	0.72
RE	2	0.96	0.71
RE	3	0.30	0.49
RE	4	0.88	2.94
RE	5	0.77	0.31
RE	6	1.12	1.44
RE	7	1.15	0.73
RE	8	Insufficient sample	Insufficient sample
	Mean	**1.00**	**1.05**
	SD	**0.46**	**0.90**

Trial	Participant	POST	+3 h
HIT+RE	1	0.17	1.29
HIT+RE	2	0.62	0.82
HIT+RE	3	0.46	1.44
HIT+RE	4	0.72	1.48
HIT+RE	5	3.21	2.84
HIT+RE	6	0.84	0.74
HIT+RE	7	0.02	0.32
HIT+RE	8	Insufficient sample	Insufficient sample
	Mean	**0.86**	**1.27**
	SD	**1.07**	**0.81**

Trial	Participant	POST	+3 h
MICT+RE	1	0.28	0.73
MICT+RE	2	0.63	1.14
MICT+RE	3	0.78	1.36
MICT+RE	4	0.18	0.92
MICT+RE	5	0.63	1.30
MICT+RE	6	0.12	0.34
MICT+RE	7	1.25	0.11
MICT+RE	8	Insufficient sample	Insufficient sample
	Mean	**0.55**	**0.84**
	SD	**0.40**	**0.48**

Study 1: qPCR microRNA data

miR-1
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	0.09	1.30	0.26
RE	2	1.55	1.50	1.38
RE	3	1.64	1.90	1.77
RE	4	1.13	1.04	0.84
RE	5	0.69	0.65	0.70
RE	6	1.02	1.18	1.03
RE	7	0.68	1.11	0.88
RE	8	1.20	1.31	1.53
	Mean	**1.00**	**1.25**	**1.05**
	SD	**0.51**	**0.36**	**0.49**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	2.08	2.02	1.91
HIT+RE	2	0.77	0.24	1.26
HIT+RE	3	1.54	1.34	1.22
HIT+RE	4	0.66	0.21	0.36
HIT+RE	5	0.77	1.13	1.03
HIT+RE	6	1.00	0.87	1.34
HIT+RE	7	1.72	1.23	0.96
HIT+RE	8	0.44	0.54	0.87
	Mean	**1.12**	**0.95**	**1.12**
	SD	**0.58**	**0.61**	**0.44**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	1.37	0.42	1.62
MICT+RE	2	0.98	0.69	0.62
MICT+RE	3	2.35	2.89	2.75
MICT+RE	4	0.93	1.90	1.21
MICT+RE	5	1.24	0.91	1.05
MICT+RE	6	0.22	0.60	0.92
MICT+RE	7	1.01	0.66	0.89
MICT+RE	8	1.26	1.45	1.67
	Mean	**1.17**	**1.19**	**1.34**
	SD	**0.59**	**0.85**	**0.67**

miR-133a
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	3.79	0.89	1.71
RE	2	0.51	0.48	0.29
RE	3	0.97	0.86	0.69
RE	4	0.45	0.38	0.34
RE	5	0.36	0.30	0.32
RE	6	0.54	0.58	0.56
RE	7	0.70	0.48	0.40
RE	8	0.68	0.69	0.77
	Mean	**1.00**	**0.58**	**0.63**
	SD	**1.14**	**0.22**	**0.47**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	1.04	0.77	0.85
HIT+RE	2	0.35	0.11	0.35
HIT+RE	3	0.59	0.53	0.49
HIT+RE	4	0.28	0.23	0.32
HIT+RE	5	0.33	0.42	0.46
HIT+RE	6	0.46	0.47	0.57
HIT+RE	7	0.60	0.46	0.19
HIT+RE	8	0.22	0.29	0.75
	Mean	**0.49**	**0.41**	**0.50**
	SD	**0.26**	**0.21**	**0.22**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	0.79	0.62	0.56
MICT+RE	2	0.58	0.56	0.41
MICT+RE	3	0.87	0.85	0.71
MICT+RE	4	0.55	0.73	0.48
MICT+RE	5	0.50	0.41	0.28
MICT+RE	6	0.46	0.55	0.43
MICT+RE	7	0.56	0.52	0.41
MICT+RE	8	0.76	0.87	0.74
	Mean	**0.64**	**0.64**	**0.50**
	SD	**0.15**	**0.16**	**0.16**

miR-378

Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	2.55	0.96	1.51
RE	2	0.78	0.62	0.64
RE	3	0.97	1.26	1.20
RE	4	0.64	0.64	0.68
RE	5	0.41	0.41	0.47
RE	6	0.79	0.69	0.72
RE	7	0.86	0.82	0.84
RE	8	1.00	0.86	0.99
	Mean	**1.00**	**0.78**	**0.88**
	SD	**0.65**	**0.26**	**0.34**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	1.00	0.86	1.04
HIT+RE	2	0.46	0.16	0.71
HIT+RE	3	0.59	0.62	0.65
HIT+RE	4	0.45	0.34	0.57
HIT+RE	5	0.41	0.49	0.54
HIT+RE	6	0.67	0.61	0.69
HIT+RE	7	1.06	0.90	0.81
HIT+RE	8	0.31	0.30	0.92
	Mean	**0.62**	**0.54**	**0.74**
	SD	**0.28**	**0.26**	**0.17**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	1.11	0.92	1.05
MICT+RE	2	0.42	0.52	0.40
MICT+RE	3	1.27	1.56	1.37
MICT+RE	4	0.54	0.80	0.58
MICT+RE	5	0.71	0.63	0.61
MICT+RE	6	0.64	0.64	0.75
MICT+RE	7	0.82	0.63	0.61
MICT+RE	8	1.09	1.29	1.66
	Mean	**0.82**	**0.87**	**0.88**
	SD	**0.30**	**0.37**	**0.44**

miR-486
Data expressed as fold difference from PRE (AU)

Trial	Participant	PRE	+1 h	+3 h
RE	1	1.71	0.80	1.22
RE	2	0.86	0.99	0.85
RE	3	0.86	1.27	1.01
RE	4	0.71	1.15	0.75
RE	5	0.49	1.11	0.76
RE	6	0.84	0.73	0.70
RE	7	1.16	0.93	0.79
RE	8	1.37	1.23	1.48
	Mean	**1.00**	**1.03**	**0.94**
	SD	**0.39**	**0.20**	**0.28**

Trial	Participant	POST	+1 h	+3 h
HIT+RE	1	0.83	0.88	0.93
HIT+RE	2	0.58	0.33	0.78
HIT+RE	3	0.61	0.59	0.55
HIT+RE	4	0.56	0.70	0.95
HIT+RE	5	0.47	0.49	0.66
HIT+RE	6	0.70	0.79	0.70
HIT+RE	7	1.12	1.15	1.21
HIT+RE	8	0.39	0.56	1.12
	Mean	**0.66**	**0.69**	**0.86**
	SD	**0.23**	**0.25**	**0.23**

Trial	Participant	POST	+1 h	+3 h
MICT+RE	1	1.64	1.71	1.18
MICT+RE	2	0.48	0.58	0.51
MICT+RE	3	1.35	1.53	2.50
MICT+RE	4	0.54	0.99	0.59
MICT+RE	5	0.84	0.88	0.86
MICT+RE	6	0.44	0.77	0.59
MICT+RE	7	1.03	0.94	0.70
MICT+RE	8	1.29	1.51	1.45
	Mean	**0.95**	**1.11**	**1.05**
	SD	**0.45**	**0.41**	**0.67**

Study 1: Physiological and psychological data from single-bout experimental trials

Venous blood lactate (mmol·L^{-1})

		Time point during exercise																	
Trial	**Participant**	**Rest**	**Pre**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
HIT+RE	1	0.3	0.5	1.9	3.3	3.8	4.6	4.2	5.1	4.9	4.1	3.3	1.7	2.2	1.8	1.4	0.9	0.6	0.7	0.6
HIT+RE	2	0.7	0.7	4.4	6.3	7.8	8.4	9.2	8.2	8.6	8.3	7.2	3.1	3.0	2.9	2.4	1.7	1.0	0.9	0.6
HIT+RE	3	0.6	0.9	3.9	6.0	7.3	8.1	8.4	7.7	8.0	7.1	6.4	2.7	3.0	2.6	2.2	1.5	1.1	1.0	0.7
HIT+RE	4	0.8	1.0	2.5	3.7	4.0	4.2	4.4	4.4	4.2	3.6	3.1	2.5	3.0	2.5	1.7	1.1	0.8	0.7	0.6
HIT+RE	5	0.5	0.7	2.3	3.6	4.9	5.7	6.9	6.7	6.5	5.6	4.5	1.3	1.4	1.4	1.3	0.8	0.7	0.6	0.5
HIT+RE	6	0.8	1.0	3.7	5.8	6.8	7.0	7.2	7.3	6.7	6.0	4.6	3.5	3.5	3.4	2.8	1.5	1.2	1.0	0.8
HIT+RE	7	0.5	0.8	2.1	3.2	4.5	4.5	4.5	3.8	3.3	2.2	1.7	1.2	1.2	1.1	0.9	0.5	0.8	0.4	0.4
HIT+RE	8	0.8	0.8	1.9	2.7	3.4	3.3	4.2	3.7	2.5	2.3	1.7	1.6	1.4	1.2	1.2	0.7	0.6	0.4	0.4
	Mean	**0.6**	**0.8**	**2.8**	**4.3**	**5.3**	**5.7**	**6.1**	**5.8**	**5.6**	**4.9**	**4.1**	**2.2**	**2.3**	**2.1**	**1.7**	**1.1**	**0.9**	**0.7**	**0.6**
	SD	**0.2**	**0.2**	**1.0**	**1.5**	**1.7**	**1.9**	**2.0**	**1.8**	**2.2**	**2.2**	**2.0**	**0.9**	**0.9**	**0.8**	**0.7**	**0.4**	**0.2**	**0.2**	**0.1**

		Time point during exercise																	
Trial	**Participant**	**Rest**	**Pre**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
MICT+RE	1	0.4	0.8	1.7	2.4	2.6	2.5	2.7	2.7	2.5	1.8	1.7	1.5	1.4	1.4	1.2	N/A	1.2	N/A	0.6
MICT+RE	2	0.4	0.5	1.2	1.6	1.8	2.3	2.2	1.7	1.6	1.5	1.4	1.2	1.3	1.5	1.4	0.9	0.7	0.7	0.5
MICT+RE	3	0.6	0.8	1.4	1.6	1.6	1.7	1.6	1.8	1.6	1.4	1.2	1.9	1.8	1.6	1.5	0.9	0.7	0.6	0.5
MICT+RE	4	0.6	1.2	1.9	2.0	2.3	2.3	2.1	2.1	2.0	1.4	1.2	3.4	3.4	2.0	2.3	1.2	0.9	0.9	0.8
MICT+RE	5	0.6	1.0	1.6	2.9	3.4	3.4	3.3	3.2	2.9	2.2	1.8	1.3	1.1	1.1	0.9	0.7	0.5	0.5	0.5
MICT+RE	6	0.9	0.8	1.8	2.7	2.9	3.1	3.4	2.9	2.6	2.3	N/A	1.7	1.8	1.8	1.8	1.1	1.0	0.8	0.7
MICT+RE	7	0.6	1.0	2.2	2.3	2.3	2.3	2.6	2.1	1.8	1.6	1.3	1.5	1.3	1.2	1.1	0.7	0.6	0.6	0.5
MICT+RE	8	0.5	0.9	1.2	1.4	1.4	1.5	1.5	1.5	1.3	1.2	1.3	1.5	1.4	1.2	1.1	0.9	0.8	0.7	0.6
	Mean	**0.6**	**0.9**	**1.6**	**2.1**	**2.3**	**2.4**	**2.4**	**2.2**	**2.0**	**1.6**	**1.4**	**1.7**	**1.7**	**1.5**	**1.4**	**0.9**	**0.8**	**0.7**	**0.6**
	SD	**0.2**	**0.2**	**0.4**	**0.6**	**0.7**	**0.6**	**0.7**	**0.6**	**0.6**	**0.4**	**0.2**	**0.7**	**0.7**	**0.3**	**0.4**	**0.2**	**0.2**	**0.1**	**0.1**

		Time point during exercise											
Trial	**Participant**	**Rest**	**+45 min**	**+80 min**	**+95 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
RT only	1	0.7	0.9	0.8	N/A	1.6	1.5	1.5	1.3	0.8	0.7	0.6	N/A
RT only	2	0.7	0.8	0.7	0.7	1.2	1.3	1.1	1.1	0.9	0.6	0.7	0.4
RT only	3	0.6	0.8	0.7	0.8	2.3	1.6	1.6	1.5	1.3	0.9	0.8	0.9
RT only	4	0.7	1.1	0.8	0.7	3.2	3.5	2.8	2.0	0.9	0.8	0.8	N/A
RT only	5	0.7	1.1	0.8	0.7	1.3	1.2	N/A	0.8	0.7	0.5	0.5	0.5
RT only	6	0.7	0.9	0.9	0.9	1.7	1.6	1.6	1.4	0.7	0.6	0.7	0.6
RT only	7	0.5	1.0	0.7	0.8	1.1	1.2	0.9	0.9	0.6	0.5	0.5	0.4
RT only	8	0.7	1.0	0.6	0.6	1.0	N/A	1.0	0.8	0.5	0.5	0.4	0.4
	Mean	**0.7**	**1.0**	**0.7**	**0.8**	**1.7**	**1.7**	**1.5**	**1.2**	**0.8**	**0.6**	**0.6**	**0.5**
	SD	**0.1**	**0.1**	**0.1**	**0.1**	**0.7**	**0.8**	**0.6**	**0.4**	**0.2**	**0.2**	**0.2**	**0.2**

Venous blood glucose (mmol·L^{-1})

		Time point during exercise																	
Trial	**Participant**	**Rest**	**Pre**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
HIT+RE	1	5.3	5.9	3.9	4.1	4.7	5.2	5.4	6.2	5.8	5.5	3.9	4.4	4.5	4.2	4.7	4.4	4.2	4.1	4.1
HIT+RE	2	3.7	3.9	3.1	3.1	3.6	3.8	4.2	4.6	4.6	4.4	4.0	4.2	4.3	4.4	4.2	4.1	4.4	4.3	4.3
HIT+RE	3	3.7	4.7	3.2	3.5	4.0	4.4	4.8	5.6	5.1	4.7	4.4	3.2	3.8	3.8	3.7	3.8	4.1	4.1	3.9
HIT+RE	4	4.5	3.9	3.1	3.7	4.1	4.4	4.5	5.0	4.9	4.8	4.8	4.3	4.5	4.4	3.5	4.5	4.4	4.6	4.5
HIT+RE	5	5.3	7.9	6.2	5.2	5.0	5.0	5.4	6.0	5.2	5.2	5.1	3.8	4.0	3.9	3.8	3.9	3.9	4.3	4.3
HIT+RE	6	3.7	2.7	3.2	3.6	3.8	4.0	4.1	4.3	4.2	4.0	3.8	3.8	4.0	4.1	3.9	3.8	3.7	3.9	4.1
HIT+RE	7	4.1	3.0	2.1	3.2	3.8	4.1	4.2	4.4	4.4	4.1	4.1	4.5	4.6	4.4	4.4	4.7	4.7	4.7	4.7
HIT+RE	8	4.6	2.1	2.5	3.1	3.7	3.8	4.2	4.2	4.3	4.1	4.4	4.3	4.3	4.3	4.6	4.3	4.4	4.5	4.4
	Mean	**4.4**	**4.2**	**3.4**	**3.7**	**4.1**	**4.3**	**4.6**	**5.0**	**4.8**	**4.6**	**4.3**	**4.1**	**4.2**	**4.2**	**4.1**	**4.2**	**4.2**	**4.3**	**4.3**
	SD	**0.7**	**1.9**	**1.2**	**0.7**	**0.5**	**0.5**	**0.5**	**0.8**	**0.5**	**0.5**	**0.4**	**0.4**	**0.3**	**0.2**	**0.4**	**0.3**	**0.3**	**0.3**	**0.3**

		Time point during exercise																	
Trial	**Participant**	**Rest**	**Pre**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
MICT+RE	1	4.5	5.4	3.9	3.6	3.5	3.8	4.1	4.3	4.4	4.0	4.2	4.4	4.4	4.5	3.8	N/A	4.2	N/A	4.4
MICT+RE	2	4.6	5.4	3.9	3.6	3.6	3.8	3.2	3.2	3.1	3.2	3.3	3.9	3.8	3.7	3.7	4.1	4.0	4.1	4.2
MICT+RE	3	4.2	4.8	4.1	3.4	3.2	3.2	3.1	4.0	4.0	4.0	4.3	4.5	4.6	4.5	4.6	4.6	4.3	4.0	4.3
MICT+RE	4	4.6	4.5	3.2	3.7	4.3	4.4	4.6	4.3	4.6	4.8	5.0	4.7	4.7	3.6	4.7	4.5	4.6	4.7	4.5
MICT+RE	5	4.3	5.8	4.3	3.4	3.2	3.3	3.3	4.1	3.9	3.6	3.6	4.1	4.2	4.2	4.2	4.3	4.3	4.2	4.3
MICT+RE	6	3.9	4.5	3.2	3.2	3.1	3.3	3.7	3.7	3.8	4.0	N/A	4.0	4.0	4.1	4.0	4.1	3.9	4.1	4.2
MICT+RE	7	4.2	4.0	3.8	3.8	3.9	4.1	4.2	4.5	4.2	4.1	4.0	4.6	4.3	4.5	4.5	4.4	4.3	4.3	4.2
MICT+RE	8	4.5	2.6	2.6	2.9	3.4	3.9	4.2	4.4	4.2	4.2	4.0	4.7	4.7	4.3	4.5	4.6	4.5	4.8	4.9
	Mean	**4.4**	**4.6**	**3.6**	**3.4**	**3.5**	**3.7**	**3.8**	**4.1**	**4.0**	**4.0**	**4.1**	**4.3**	**4.3**	**4.2**	**4.2**	**4.4**	**4.3**	**4.3**	**4.4**
	SD	**0.2**	**1.0**	**0.5**	**0.3**	**0.4**	**0.4**	**0.6**	**0.4**	**0.5**	**0.5**	**0.5**	**0.3**	**0.3**	**0.4**	**0.4**	**0.2**	**0.2**	**0.3**	**0.2**

		Time point during exercise											
Trial	**Participant**	**Rest**	**+45 min**	**+80 min**	**+95 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
RT only	1	4.3	4.5	3.3	N/A	4.0	3.9	3.9	3.9	4.0	3.9	4.1	N/A
RT only	2	4.6	5.3	4.2	4.4	4.7	4.6	4.6	4.5	4.3	4.4	4.6	4.5
RT only	3	4.4	3.5	3.1	3.7	4.6	3.0	4.4	4.3	4.0	5.7	4.9	4.7
RT only	4	4.8	4.3	3.8	3.9	4.6	4.5	4.4	4.3	4.2	4.5	4.5	N/A
RT only	5	4.7	6.2	4.2	3.7	4.0	3.7	N/A	3.8	3.9	4.0	4.2	4.2
RT only	6	3.9	4.7	4.5	4.7	4.5	4.4	4.3	4.3	4.4	4.4	4.1	4.4
RT only	7	4.5	3.5	3.7	3.7	4.5	4.7	4.5	4.8	4.7	4.0	4.4	4.4
RT only	8	4.5	3.1	4.2	4.6	4.7	N/A	4.5	4.7	4.7	4.7	4.7	4.5
	Mean	**4.5**	**4.4**	**3.9**	**4.1**	**4.4**	**4.1**	**4.4**	**4.3**	**4.3**	**4.4**	**4.4**	**4.5**
	SD	**0.3**	**1.0**	**0.5**	**0.4**	**0.3**	**0.6**	**0.2**	**0.3**	**0.3**	**0.6**	**0.3**	**0.2**

Heart rate (beats·min^{-1})

		Time point during cycling					
Trial	**Participant**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
HIT+RE	1	72	152	171	178	183	184
HIT+RE	2	45	151	160	165	168	172
HIT+RE	3	67	176	185	188	190	191
HIT+RE	4	78	165	168	170	174	175
HIT+RE	5	75	167	176	180	185	186
HIT+RE	6	51	156	160	163	165	172
HIT+RE	7	56	156	162	167	171	174
HIT+RE	8	62	160	164	169	169	172
	Mean	**63**	**160**	**168**	**173**	**176**	**178**
	SD	**12**	**9**	**9**	**9**	**9**	**8**

		Time point during cycling					
Trial	**Participant**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
MICT+RE	1	67	117	132	137	143	144
MICT+RE	2	45	110	120	128	128	134
MICT+RE	3	61	129	138	142	147	145
MICT+RE	4	79	128	138	146	146	144
MICT+RE	5	68	135	158	165	165	167
MICT+RE	6	56	114	125	135	134	130
MICT+RE	7	56	119	127	132	134	137
MICT+RE	8	62	129	125	125	130	128
	Mean	**62**	**123**	**133**	**139**	**141**	**141**
	SD	**10**	**9**	**12**	**13**	**12**	**12**

Rating of perceived exertion (RPE [AU]**)**

		Time point during cycling					
Trial	**Participant**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
HIT+RE	1	6	15	16	17	18	18
HIT+RE	2	6	13	14	15	17	17
HIT+RE	3	6	17	19	19	20	20
HIT+RE	4	6	13	14	14	15	17
HIT+RE	5	6	14	15	16	17	18
HIT+RE	6	6	15	15	16	17	18
HIT+RE	7	6	12	13	14	15	16
HIT+RE	8	6	13	14	15	16	17
	Mean	**6**	**14**	**15**	**16**	**17**	**18**
	SD	**0**	**2**	**2**	**2**	**2**	**1**

		Time point during cycling					
Trial	**Participant**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
MICT+RE	1	6	9	9	10	11	11
MICT+RE	2	6	12	12	13	13	13
MICT+RE	3	6	10	13	14	15	15
MICT+RE	4	6	8	10	11	11	11
MICT+RE	5	6	11	13	14	15	15
MICT+RE	6	6	9	9	9	9	9
MICT+RE	7	6	9	11	11	11	11
MICT+RE	8	6	10	12	13	14	15
	Mean	**6**	**10**	**11**	**12**	**12**	**13**
	SD	**0**	**1**	**2**	**2**	**2**	**2**

Appendix L: Raw data for Study 2 (Chapters 4 and 5)

Study 2: Maximal (1-RM) strength data

1-RM leg press (kg)

Group	Participant	PRE	MID	POST	Δ PRE (kg)
RT only	1	353	408	433	80.0
RT only	2	253	325	428	175.0
RT only	3	293	328	393	100.0
RT only	4	353	394	423	70.0
RT only	5	363	383	438	75.0
RT only	6	323	388	473	150.0
RT only	7	193	253	293	100.0
RT only	8	273	323	413	140.0
	Mean	**300.5**	**350.3**	**411.8**	**111.3**
	SD	**59.2**	**52.2**	**53.1**	**39.0**

Group	Participant	PRE	MID	POST	Δ PRE (kg)
HIT+RT	1	300	328	353	53.0
HIT+RT	2	213	238	278	65.0
HIT+RT	3	323	398	423	100.0
HIT+RT	4	393	423	463	70.0
HIT+RT	5	283	313	358	75.0
HIT+RT	6	238	283	343	105.0
HIT+RT	7	323	373	423	100.0
HIT+RT	8	318	368	423	105.0
	Mean	**298.9**	**340.5**	**383.0**	**84.1**
	SD	**55.7**	**61.7**	**60.2**	**20.7**

Group	Participant	PRE	MID	POST	Δ PRE (kg)
MICT+RT	1	173	203	263	90.0
MICT+RT	2	383	403	438	55.0
MICT+RT	3	278	333	348	70.0
MICT+RT	4	245.5	293	343	97.5
MICT+RT	5	303	353	348	45.0
MICT+RT	6	343	388	428	85.0
MICT+RT	7	313	373	393	80.0
	Mean	**291.2**	**335.1**	**365.9**	**74.6**
	SD	**68.3**	**68.8**	**59.9**	**19.1**

1-RM bench press (kg)

Group	Participant	PRE	MID	POST	Δ PRE (kg)
RT only	1	90	107.5	115	25.0
RT only	2	62.5	Did not test	72.5	10.0
RT only	3	70	82.5	90	20.0
RT only	4	60	72.5	75	15.0
RT only	5	92.5	95	95	2.5
RT only	6	92.5	100	102.5	10.0
RT only	7	35	42.5	45	10.0
RT only	8	55	60	70	15.0
	Mean	**69.7**	**80.0**	**83.1**	**13.4**
	SD	**20.8**	**23.3**	**22.0**	**6.9**

Group	Participant	PRE	MID	POST	Δ PRE (kg)
HIT+RT	1	80	85	90	10.0
HIT+RT	2	52.5	57.5	67.5	15.0
HIT+RT	3	80	82.5	87.5	7.5
HIT+RT	4	100	107.5	115	15.0
HIT+RT	5	90	95	102.5	12.5
HIT+RT	6	80	90	93	13.0
HIT+RT	7	85	95	95	10.0
HIT+RT	8	60	67.5	72.5	12.5
	Mean	**78.4**	**85.0**	**90.4**	**11.9**
	SD	**15.4**	**16.0**	**15.3**	**2.6**

Group	Participant	PRE	MID	POST	Δ PRE (kg)
MICT+RT	1	50	55	60	10.0
MICT+RT	2	125	120	130	5.0
MICT+RT	3	65	70	75	10.0
MICT+RT	4	77.5	88	90	12.5
MICT+RT	5	60	67.5	72.5	12.5
MICT+RT	6	92.5	97.5	107.5	15.0
MICT+RT	7	85	90	95	10.0
	Mean	**79.3**	**84.0**	**90.0**	**10.7**
	SD	**24.9**	**21.8**	**23.7**	**3.1**

Study 2: Counter-movement jump (CMJ) data

Peak CMJ force (N)

Group	Participant	PRE	MID	POST	Δ PRE (N)
RT only	1	2307.1	2355.4	2347.6	40.5
RT only	2	1597.0	1727.4	1809.9	212.8
RT only	3	1898.5	1854.6	1975.7	77.3
RT only	4	1794.6	1927.5	2097.6	303.0
RT only	5	2145.2	2160.5	2186.3	41.1
RT only	6	1837.2	1793.0	1918.4	81.2
RT only	7	1583.4	1563.5	1655.6	72.2
RT only	8	1611.6	1715.6	1828.3	216.7
	Mean	**1846.8**	**1887.2**	**1977.4**	**130.6**
	SD	**265.5**	**257.8**	**224.4**	**99.1**

Group	Participant	PRE	MID	POST	Δ PRE (N)
HIT+RT	1	1992.5	2039.3	2069.9	77.4
HIT+RT	2	1375.7	1409.3	1295.5	-80.2
HIT+RT	3	1832.5	1655.3	1792.0	-40.5
HIT+RT	4	1944.4	2000.4	1999.2	54.8
HIT+RT	5	1790.5	Did not test	1947.2	156.7
HIT+RT	6	1468.9	1564.4	1432.2	-36.7
HIT+RT	7	1853.7	1818.5	1915.7	62.1
HIT+RT	8	1957.4	1963.1	1823.5	-133.9
	Mean	**1777.0**	**1778.6**	**1784.4**	**7.4**
	SD	**230.6**	**241.6**	**276.7**	**95.9**

Group	Participant	PRE	MID	POST	Δ PRE (N)
MICT+RT	1	1419.3	1377.0	1464.6	45.3
MICT+RT	2	2419.4	2293.8	2104.0	-315.4
MICT+RT	3	1850.1	1827.4	1827.4	-22.7
MICT+RT	4	2115.4	2116.3	2044.7	-70.7
MICT+RT	5	1810.6	1808.2	2044.7	234.1
MICT+RT	6	1693.1	1616.0	1710.1	17.0
MICT+RT	7	1796.3	1658.1	1734.2	-62.1
	Mean	**1872.0**	**1813.8**	**1847.1**	**-24.9**
	SD	**317.7**	**309.9**	**231.8**	**164.1**

Peak CMJ power (W)

Group	Participant	PRE	MID	POST	Δ PRE (W)
RT only	1	2498.8	2655.0	2980.7	482.0
RT only	2	2997.0	3002.0	3474.5	477.5
RT only	3	2461.1	2566.8	2565.9	104.8
RT only	4	2874.5	2796.8	4123.2	1248.7
RT only	5	3227.2	3043.0	3288.7	61.5
RT only	6	3093.4	2930.7	3045.7	-47.7
RT only	7	2828.3	2853.9	2868.2	39.9
RT only	8	2702.1	3158.5	3320.2	618.2
	Mean	**2835.3**	**2875.8**	**3208.4**	**373.1**
	SD	**272.3**	**199.2**	**468.0**	**432.0**

Group	Participant	PRE	MID	POST	Δ PRE (W)
HIT+RT	1	2587.1	2920.0	2933.7	346.6
HIT+RT	2	2226.3	2332.0	2004.1	-222.2
HIT+RT	3	2714.6	2724.2	2710.5	-4.1
HIT+RT	4	3051.9	3092.5	3282.7	230.8
HIT+RT	5	2348.4	Did not test	2605.3	257.0
HIT+RT	6	2407.0	2370.8	2471.3	64.3
HIT+RT	7	2949.8	2872.5	2882.8	-67.0
HIT+RT	8	3309.8	3315.9	3501.5	191.7
	Mean	**2699.3**	**2804.0**	**2799.0**	**99.6**
	SD	**378.9**	**360.6**	**468.6**	**190.8**

Group	Participant	PRE	MID	POST	Δ PRE (W)
MICT+RT	1	2096.3	2569.2	2677.4	581.1
MICT+RT	2	3942.7	3791.4	3739.4	-203.3
MICT+RT	3	2796.7	2856.1	2856.1	59.4
MICT+RT	4	3481.7	3254.6	3588.2	106.5
MICT+RT	5	2312.5	2438.6	2300.5	-12.0
MICT+RT	6	2692.9	2642.9	2987.2	294.3
MICT+RT	7	3097.8	3086.5	3092.6	-5.2
	Mean	**2917.2**	**2948.5**	**3034.5**	**117.3**
	SD	**646.4**	**470.6**	**501.2**	**253.1**

Peak CMJ velocity ($m \cdot s^{-1}$)

Group	Participant	PRE	MID	POST	Δ PRE ($m \cdot s^{-1}$)
RT only	1	1.10	1.29	1.46	0.36
RT only	2	2.02	1.89	2.24	0.22
RT only	3	1.61	1.63	1.59	-0.03
RT only	4	1.75	1.68	2.32	0.57
RT only	5	1.92	1.89	1.97	0.05
RT only	6	2.43	2.41	2.31	-0.12
RT only	7	2.25	2.20	2.16	-0.09
RT only	8	1.98	2.19	2.31	0.33
	Mean	**1.88**	**1.90**	**2.04**	**0.16**
	SD	**0.41**	**0.36**	**0.34**	**0.25**

Group	Participant	PRE	MID	POST	Δ PRE ($m \cdot s^{-1}$)
HIT+RT	1	1.72	1.72	1.73	0.01
HIT+RT	2	1.81	1.86	1.74	-0.07
HIT+RT	3	1.75	1.77	1.73	-0.02
HIT+RT	4	1.70	1.69	1.82	0.12
HIT+RT	5	1.62	Did not test	1.88	0.26
HIT+RT	6	1.82	1.75	1.93	0.10
HIT+RT	7	1.76	1.74	1.68	-0.08
HIT+RT	8	2.21	2.20	2.28	0.07
	Mean	**1.80**	**1.82**	**1.85**	**0.05**
	SD	**0.18**	**0.18**	**0.19**	**0.11**

Group	Participant	PRE	MID	POST	Δ PRE ($m \cdot s^{-1}$)
MICT+RT	1	1.68	1.88	1.86	0.18
MICT+RT	2	1.85	1.86	1.93	0.08
MICT+RT	3	1.71	1.72	1.72	0.01
MICT+RT	4	1.92	1.83	2.16	0.24
MICT+RT	5	1.62	1.69	1.70	0.08
MICT+RT	6	1.86	1.88	2.03	0.17
MICT+RT	7	2.15	2.09	2.16	0.00
	Mean	**1.83**	**1.85**	**1.94**	**0.11**
	SD	**0.18**	**0.13**	**0.19**	**0.09**

Peak CMJ displacement (m)

Group	Participant	PRE	MID	POST	Δ PRE (m)
RT only	1	0.277	0.299	0.321	0.045
RT only	2	0.454	0.431	0.594	0.140
RT only	3	0.375	0.382	0.371	-0.004
RT only	4	0.387	0.439	0.464	0.077
RT only	5	0.522	0.525	0.545	0.023
RT only	6	0.665	0.646	0.610	-0.055
RT only	7	0.637	0.628	0.597	-0.040
RT only	8	0.494	0.560	0.634	0.140
	Mean	**0.476**	**0.489**	**0.517**	**0.041**
	SD	**0.132**	**0.122**	**0.118**	**0.075**

Group	Participant	PRE	MID	POST	Δ PRE (m)
HIT+RT	1	0.394	0.454	0.437	0.042
HIT+RT	2	0.467	0.464	0.443	-0.023
HIT+RT	3	0.441	0.440	0.434	-0.007
HIT+RT	4	0.408	0.411	0.435	0.027
HIT+RT	5	0.349	Did not test	0.487	0.138
HIT+RT	6	0.439	0.407	0.477	0.038
HIT+RT	7	0.410	0.420	0.392	-0.018
HIT+RT	8	0.550	0.565	0.625	0.076
	Mean	**0.432**	**0.451**	**0.466**	**0.034**
	SD	**0.059**	**0.054**	**0.071**	**0.054**

Group	Participant	PRE	MID	POST	Δ PRE (m)
MICT+RT	1	0.416	0.447	0.434	0.019
MICT+RT	2	0.468	0.465	0.489	0.021
MICT+RT	3	0.460	0.429	0.429	-0.031
MICT+RT	4	0.493	0.475	0.614	0.121
MICT+RT	5	0.359	0.420	0.417	0.058
MICT+RT	6	0.494	0.482	0.555	0.061
MICT+RT	7	0.633	0.580	0.614	-0.019
	Mean	**0.475**	**0.471**	**0.507**	**0.033**
	SD	**0.085**	**0.053**	**0.087**	**0.052**

Study 2: Graded exercise testing (GXT) data

Absolute $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)

Group	Participant	PRE	MID	POST	Δ PRE ($L \cdot min^{-1}$)
RT only	1	2.93	3.11	2.74	-0.19
RT only	2	4.32	3.78	4.46	0.14
RT only	3	3.16	3.54	3.58	0.43
RT only	4	2.75	3.55	2.82	0.07
RT only	5	3.55	Did not test	3.66	0.11
RT only	6	4.40	4.06	3.60	-0.80
RT only	7	2.37	2.51	2.59	0.23
RT only	8	4.18	3.91	3.84	-0.34
	Mean	**3.46**	**3.49**	**3.41**	**-0.05**
	SD	**0.78**	**0.53**	**0.64**	**0.39**

Group	Participant	PRE	MID	POST	Δ PRE ($L \cdot min^{-1}$)
HIT+RT	1	3.19	3.35	3.50	0.31
HIT+RT	2	4.57	4.31	4.45	-0.12
HIT+RT	3	3.47	3.37	3.69	0.23
HIT+RT	4	3.35	3.55	3.99	0.64
HIT+RT	5	3.63	3.95	3.86	0.23
HIT+RT	6	3.86	3.61	4.07	0.20
HIT+RT	7	3.09	3.61	3.49	0.40
HIT+RT	8	5.24	4.84	4.61	-0.63
	Mean	**3.80**	**3.82**	**3.96**	**0.16**
	SD	**0.75**	**0.52**	**0.41**	**0.38**

Group	Participant	PRE	MID	POST	Δ PRE ($L \cdot min^{-1}$)
MICT+RT	1	3.65	3.91	3.86	0.21
MICT+RT	2	3.44	3.74	3.53	0.10
MICT+RT	3	4.03	5.70	3.76	-0.27
MICT+RT	4	3.30	3.08	3.67	0.37
MICT+RT	5	3.88	4.53	4.37	0.49
MICT+RT	6	3.10	3.47	3.71	0.60
MICT+RT	7	4.09	4.30	4.09	0.00
	Mean	**3.64**	**4.10**	**3.86**	**0.21**
	SD	**0.38**	**0.85**	**0.29**	**0.30**

Relative $\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)

Group	Participant	PRE	MID	POST	Δ **PRE** ($mL \cdot kg^{-1} \cdot min^{-1}$)
RT only	1	25.8	27.3	23.9	-1.9
RT only	2	53.0	45.7	53.0	0.0
RT only	3	34.4	38.0	38.2	3.8
RT only	4	30.5	34.7	33.2	2.7
RT only	5	41.2	Did not test	41.9	0.6
RT only	6	61.5	56.3	49.0	-12.6
RT only	7	37.2	38.8	39.0	1.8
RT only	8	53.8	48.9	47.6	-6.3
	Mean	**42.2**	**41.3**	**40.7**	**-1.5**
	SD	**12.6**	**8.9**	**9.4**	**5.4**

Group	Participant	PRE	MID	POST	Δ **PRE** ($mL \cdot kg^{-1} \cdot min^{-1}$)
HIT+RT	1	34.1	35.5	37.5	3.4
HIT+RT	2	68.2	64.5	64.9	-3.3
HIT+RT	3	46.9	49.9	49.1	2.3
HIT+RT	4	35.0	37.4	42.6	7.6
HIT+RT	5	42.2	46.0	44.2	2.0
HIT+RT	6	55.5	51.5	58.4	2.9
HIT+RT	7	34.2	38.2	37.1	2.8
HIT+RT	8	62.4	56.6	53.6	-8.9
	Mean	**47.3**	**47.4**	**48.4**	**1.1**
	SD	**13.4**	**10.2**	**10.0**	**5.0**

Group	Participant	PRE	MID	POST	Δ **PRE** ($mL \cdot kg^{-1} \cdot min^{-1}$)
MICT+RT	1	48.2	51.2	49.6	1.5
MICT+RT	2	32.2	36.3	34.7	2.5
MICT+RT	3	48.6	62.3	45.0	-3.7
MICT+RT	4	40.6	37.4	42.4	1.8
MICT+RT	5	47.1	54.0	52.1	5.0
MICT+RT	6	36.8	41.1	43.1	6.4
MICT+RT	7	50.1	52.4	50.9	0.8
	Mean	**43.4**	**47.8**	**45.4**	**2.0**
	SD	**6.9**	**9.7**	**6.1**	**3.2**

Lactate threshold (W)

Group	Participant	PRE	MID	POST	Δ PRE (W)
RT only	1	79	103	97	18
RT only	2	208	219	241	33
RT only	3	154	Did not test	162	8
RT only	4	137	126	133	-3
RT only	5	177	190	193	16
RT only	6	197	176	167	-31
RT only	7	88	88	92	4
RT only	8	122	169	154	32
	Mean	**145**	**153**	**155**	**10**
	SD	**48**	**48**	**49**	**21**

Group	Participant	PRE	MID	POST	Δ PRE (W)
HIT+RT	1	152	155	162	11
HIT+RT	2	271	260	273	2
HIT+RT	3	156	158	173	17
HIT+RT	4	134	150	182	48
HIT+RT	5	176	176	191	16
HIT+RT	6	177	167	186	8
HIT+RT	7	135	128	137	2
HIT+RT	8	258	262	260	2
	Mean	**182**	**182**	**196**	**13**
	SD	**53**	**51**	**47**	**15**

Group	Participant	PRE	MID	POST	Δ PRE (W)
MICT+RT	1	186	181	198	12
MICT+RT	2	109	122	153	45
MICT+RT	3	119	147	136	17
MICT+RT	4	100	128	138	38
MICT+RT	5	252	241	252	0
MICT+RT	6	155	168	169	14
MICT+RT	7	191	167	171	-21
	Mean	**159**	**165**	**174**	**15**
	SD	**55**	**40**	**40**	**22**

Peak aerobic power (W)

Group	Participant	PRE	MID	POST	Δ PRE (W)
RT only	1	189	186	208	19
RT only	2	317	338	345	28
RT only	3	240	240	248	8
RT only	4	210	193	193	-17
RT only	5	280	278	261	-19
RT only	6	294	278	247	-47
RT only	7	157	159	157	0
RT only	8	270	270	253	-17
	Mean	**245**	**243**	**239**	**-6**
	SD	**56**	**60**	**56**	**24**

Group	Participant	PRE	MID	POST	Δ PRE (W)
HIT+RT	1	235	249	254	19
HIT+RT	2	348	371	365	17
HIT+RT	3	240	255	274	34
HIT+RT	4	246	220	273	27
HIT+RT	5	259	285	318	58
HIT+RT	6	295	307	316	21
HIT+RT	7	231	248	250	19
HIT+RT	8	375	368	361	-14
	Mean	**279**	**288**	**301**	**23**
	SD	**55**	**57**	**46**	**20**

Group	Participant	PRE	MID	POST	Δ PRE (W)
MICT+RT	1	274	286	308	34
MICT+RT	2	219	195	236	16
MICT+RT	3	273	279	255	-18
MICT+RT	4	230	243	251	21
MICT+RT	5	351	349	347	-4
MICT+RT	6	250	249	270	20
MICT+RT	7	270	280	287	17
	Mean	**267**	**269**	**279**	**12**
	SD	**43**	**47**	**38**	**17**

Study 2: Dual X-ray Absorptiometry (DXA) data

Total lean mass (g)

Group	Participant	PRE	POST	Δ PRE (g)
RT only	1	71555.5	71867.9	312.4
RT only	2	63392.2	64736.4	1344.2
RT only	3	60374.2	61443.6	1069.4
RT only	4	61409.6	60125.2	-1284.4
RT only	5	66760.8	66245.5	-515.3
RT only	6	57572.0	58736.9	1164.9
RT only	7	46829.5	49102.6	2273.1
RT only	8	58966.8	61951.0	2984.2
	Mean	**60857.6**	**61776.1**	**918.6**
	SD	**7244.8**	**6582.5**	**1396.0**

Group	Participant	PRE	POST	Δ PRE (g)
HIT+RT	1	64164.6	66267.5	2102.9
HIT+RT	2	52135.0	53122.4	987.4
HIT+RT	3	54831.0	56543.2	1712.2
HIT+RT	4	67959.4	67834.7	-124.7
HIT+RT	5	64831.6	63949.4	-882.2
HIT+RT	6	54046.2	55201.7	1155.5
HIT+RT	7	58253.9	60114.3	1860.4
HIT+RT	8	64211.5	64560.7	349.2
	Mean	**60054.2**	**60949.2**	**895.1**
	SD	**5962.1**	**5505.1**	**1044.1**

Group	Participant	PRE	POST	Δ PRE (g)
MICT+RT	1	55581.9	57310.0	1728.1
MICT+RT	2	71191.7	69209.0	-1982.7
MICT+RT	3	59193.0	60777.3	1584.3
MICT+RT	4	60181.9	60593.0	411.1
MICT+RT	5	58000.8	64027.5	6026.7
MICT+RT	6	61877.6	63074.5	1196.9
MICT+RT	7	61140.3	61950.3	810.0
	Mean	**61023.9**	**62420.2**	**1396.3**
	SD	**4948.8**	**3684.9**	**2393.0**

Upper-body lean mass (g)

Group	Participant	PRE	POST	Δ PRE (g)
RT only	1	49338.1	48245.1	-1093.0
RT only	2	39790.6	41041.1	1250.5
RT only	3	40057.0	40855.7	798.7
RT only	4	40711.7	38956.0	-1755.7
RT only	5	43206.1	42647.0	-559.1
RT only	6	37149.2	37571.7	422.5
RT only	7	29979.5	30199.1	219.6
RT only	8	37337.1	38872.9	1535.8
	Mean	**39696.2**	**39798.6**	**102.4**
	SD	**5515.2**	**5082.1**	**1152.2**

Group	Participant	PRE	POST	Δ PRE (g)
HIT+RT	1	41277.2	43819.2	2542.0
HIT+RT	2	32333.9	32878.4	544.5
HIT+RT	3	35508.5	36644.7	1136.2
HIT+RT	4	42933.1	41998.3	-934.8
HIT+RT	5	42037.3	41586.6	-450.7
HIT+RT	6	36599.0	37006.9	407.9
HIT+RT	7	37585.3	38922.0	1336.7
HIT+RT	8	40641.4	40233.5	-407.9
	Mean	**38614.5**	**39136.2**	**521.7**
	SD	**3699.0**	**3531.0**	**1138.2**

Group	Participant	PRE	POST	Δ PRE (g)
MICT+RT	1	35294.0	36177.0	883.0
MICT+RT	2	46295.8	44751.8	-1544.0
MICT+RT	3	38106.4	38036.3	-70.1
MICT+RT	4	37755.6	38217.8	462.2
MICT+RT	5	36790.1	40878.6	4088.5
MICT+RT	6	40931.5	41684.4	752.9
MICT+RT	7	39695.4	39608.1	-87.3
	Mean	**39267.0**	**39907.7**	**640.7**
	SD	**3603.5**	**2826.4**	**1722.3**

Lower-body lean mass (g)

Group	Participant	PRE	POST	Δ PRE (g)
RT only	1	22217.4	23622.8	1405.4
RT only	2	23601.6	23695.3	93.7
RT only	3	20317.2	20587.9	270.7
RT only	4	20697.9	21169.2	471.3
RT only	5	23554.7	23598.5	43.8
RT only	6	20422.8	21165.2	742.4
RT only	7	16850.0	18903.5	2053.5
RT only	8	21629.7	23078.1	1448.4
	Mean	**21161.4**	**21977.6**	**816.2**
	SD	**2173.8**	**1780.5**	**738.6**

Group	Participant	PRE	POST	Δ PRE (g)
HIT+RT	1	22887.4	22448.3	-439.1
HIT+RT	2	19801.1	20244.0	442.9
HIT+RT	3	19322.5	19898.5	576.0
HIT+RT	4	25026.3	25836.4	810.1
HIT+RT	5	22794.3	22362.8	-431.5
HIT+RT	6	17447.2	18194.8	747.6
HIT+RT	7	20668.6	21192.3	523.7
HIT+RT	8	23570.1	24327.2	757.1
	Mean	**21439.7**	**21813.0**	**373.4**
	SD	**2536.5**	**2473.2**	**514.7**

Group	Participant	PRE	POST	Δ PRE (g)
MICT+RT	1	20287.9	21133.0	845.1
MICT+RT	2	24895.9	24457.2	-438.7
MICT+RT	3	21086.6	22741.0	1654.4
MICT+RT	4	22426.3	22375.2	-51.1
MICT+RT	5	21210.7	23148.9	1938.2
MICT+RT	6	20946.1	21390.1	444.0
MICT+RT	7	21444.9	22342.2	897.3
	Mean	**21756.9**	**22512.5**	**755.6**
	SD	**1525.4**	**1114.3**	**857.2**

Body fat (%)

Group	Participant	PRE	POST	Δ PRE (%)
RT only	1	30.5	30.2	-0.3
RT only	2	13.7	11.9	-1.8
RT only	3	25.5	25.3	-0.2
RT only	4	22.0	18.7	-3.3
RT only	5	13.1	15.3	2.2
RT only	6	10.1	9.6	-0.5
RT only	7	15.8	15.2	-0.6
RT only	8	14.6	14.5	-0.1
	Mean	**18.2**	**17.6**	**-0.6**
	SD	**7.1**	**6.9**	**1.6**

Group	Participant	PRE	POST	Δ PRE (%)
HIT+RT	1	21.6	20.0	-1.6
HIT+RT	2	9.8	9.5	-0.3
HIT+RT	3	17.8	15.9	-1.9
HIT+RT	4	19.4	18.7	-0.7
HIT+RT	5	16.0	17.4	1.4
HIT+RT	6	12.1	11.3	-0.8
HIT+RT	7	26.9	27.8	0.9
HIT+RT	8	12.5	13.9	1.4
	Mean	**17.0**	**16.8**	**-0.2**
	SD	**5.6**	**5.7**	**1.3**

Group	Participant	PRE	POST	Δ PRE (%)
MICT+RT	1	15.8	14.8	-1.0
MICT+RT	2	25.2	24.0	-1.2
MICT+RT	3	19.5	18.6	-0.9
MICT+RT	4	17.5	18.8	1.3
MICT+RT	5	20.2	16.9	-3.3
MICT+RT	6	18.9	18.5	-0.4
MICT+RT	7	14.6	13.6	-1.0
	Mean	**18.8**	**17.9**	**-0.9**
	SD	**3.5**	**3.4**	**1.4**

Study 2: Internal training load data

Total session internal training load (AU)

		Training week							
Group	**Participant**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
RT only	1	855	865	835	966	826	966	760	240
RT only	2	575	550	575	605	440	484	520	225
RT only	3	725	560	930	860	945	825	855	245
RT only	4	720	910	885	600	N/A	N/A	N/A	250
RT only	5	535	615	620	680	765	720	816	280
RT only	6	520	480	484	520	565	500	604	245
RT only	7	690	615	610	580	655	620	610	300
RT only	8	750	760	760	615	805	727.5	750	240
	Mean	**671.3**	**669.4**	**712.4**	**678.3**	**714.4**	**691.8**	**702.1**	**253.1**
	SD	**117.3**	**156.9**	**162.4**	**154.0**	**172.0**	**173.3**	**124.7**	**24.5**

		Training week							
Group	**Participant**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
HIT+RT	1	1556.5	1745.5	1807	1416	1776	1765.5	1963	783
HIT+RT	2	1289	1664	2025	1701	1799	1944	2079	1364
HIT+RT	3	1141	1345	1504	1260	1630	1401	1803	1071
HIT+RT	4	1065	1012	1166	1323	926	1274	1435	660
HIT+RT	5	N/A	N/A	N/A	1175	1542	1330	1800	656
HIT+RT	6	855	1128	1260	1227	1449	1657	2088	1235
HIT+RT	7	1197	1257	1332	1197	1520	1610	1778	504
HIT+RT	8	1004	1059	1329	881	1510	1694	1569	1048
	Mean	**1158.2**	**1315.8**	**1489.0**	**1272.5**	**1519.0**	**1584.4**	**1814.4**	**915.1**
	SD	**224.3**	**289.7**	**315.1**	**232.2**	**271.0**	**231.4**	**230.8**	**308.1**

		Training week							
Group	**Participant**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MICT+RT	1	1197	1385	1431	1333	1480	1659	1757	1071
MICT+RT	2	1319	733	981	822	1732	1587	1096	949
MICT+RT	3	240	1155	1161	1230	1381	1438	1402	1184
MICT+RT	4	828	1125	1260	1167	1449	1652	1808	1184
MICT+RT	5	444	697	877.5	870	944	1030	1147	690
MICT+RT	6	1274.5	1421	1539	1213	1329	1322.5	1486	948
MICT+RT	7	1040	1424	1940	1444	1690.5	1875	1766.5	1184
	Mean	**906.1**	**1134.3**	**1312.8**	**1154.1**	**1429.4**	**1509.1**	**1494.6**	**1030.0**
	SD	**422.7**	**311.4**	**361.5**	**229.7**	**261.6**	**274.5**	**297.0**	**183.4**

Cycling-only internal training load (AU)

		Training week							
Group	**Participant**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
HIT+RT	1	434	442	660.5	398	670	643	840	256
HIT+RT	2	499	627	781	595	812	960	942	554
HIT+RT	3	434	543	662	420	664	701	910	422
HIT+RT	4	276	367	607	368	580	579	633	238
HIT+RT	5	N/A	N/A	N/A	388	603	672	878	256
HIT+RT	6	414	419	540	394	696	774	1015	496
HIT+RT	7	414	468	540	414	574	701	986	438
HIT+RT	8	397	448	720	321	725	771	837	412
	Mean	**409.7**	**473.4**	**644.4**	**412.3**	**665.5**	**725.1**	**880.1**	**384.0**
	SD	**67.4**	**86.0**	**89.6**	**80.1**	**80.7**	**114.5**	**118.7**	**119.9**

		Training week							
Group	**Participant**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MICT+RT	1	276	364	450	302	470	576	630	348
MICT+RT	2	302	249	421	250	548	472	370	322
MICT+RT	3	60	234	299	322	412	451	487	290
MICT+RT	4	233	318	479	345	522	605	773	406
MICT+RT	5	158	289	302	256	351	381	592	290
MICT+RT	6	276	312	403.5	276	364	512	558.5	306
MICT+RT	7	322	419	720	486	725	835	895.5	496
	Mean	**232.4**	**312.1**	**439.2**	**319.6**	**484.6**	**547.4**	**615.1**	**351.1**
	SD	**93.0**	**64.3**	**141.8**	**81.1**	**129.8**	**147.6**	**175.1**	**75.7**

Total-study internal training load (AU)

Group	Participant	Prescribed (within-study) training load (AU)	Non-prescribed (external to study) training load (AU)	Combined training load (AU)
RT only	1	6313	3920	10233
RT only	2	3974	1040	5014
RT only	3	5945	1855	7800
RT only	4	3365	720	4085
RT only	5	5031	0	5031
RT only	6	3918	840	4758
RT only	7	4680	0	4680
RT only	8	5408	60	5468
	Mean	**4829.2**	**1054.4**	**5883.6**
	SD	**1038.5**	**1321.4**	**2077.0**

Group	Participant	Prescribed (within-study) training load (AU)	Non-prescribed (external to study) training load (AU)	Combined training load (AU)
HIT+RT	1	12813	1755	14568
HIT+RT	2	13865	655	14520
HIT+RT	3	11155	2760	13915
HIT+RT	4	8861	3500	12361
HIT+RT	5	6503	15855	22358
HIT+RT	6	10899	1590	12489
HIT+RT	7	10395	1500	11895
HIT+RT	8	10094	15493	25587
	Mean	**10573**	**5389**	**15962**
	SD	**2269.5**	**6406.3**	**5117.7**

Group	Participant	Prescribed (within-study) training load (AU)	Non-prescribed (external to study) training load (AU)	Combined training load (AU)
MICT+RT	1	11313	13260	24573
MICT+RT	2	9219	255	9474
MICT+RT	3	9191	850	10041
MICT+RT	4	10473	1740	12213
MICT+RT	5	6700	205	6905
MICT+RT	6	10533	594	11127
MICT+RT	7	12364	1590	13954
	Mean	**9970.4**	**2642.0**	**12612.4**
	SD	**1824.5**	**4720.6**	**5719.3**

Study 2: Average nutritional intake from baseline 72 h food diary

Group	Participant	Average energy intake ($kJ \cdot day^{-1}$)	Average protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Average fat intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Average CHO intake ($g \cdot kg^{-1} \cdot day^{-1}$)
RT only	1	8300.3	0.81	0.87	1.71
RT only	2	9175.6	1.32	1.13	2.44
RT only	3	6193.9	0.90	0.83	2.26
RT only	4	5300.5	0.78	0.51	1.79
RT only	5	8491.7	1.42	1.14	2.47
RT only	6	9636.9	1.80	1.38	3.50
RT only	7	6877.9	0.80	0.82	2.23
RT only	8	7501.4	1.05	1.14	2.41
	Mean	**7684.8**	**1.11**	**0.98**	**2.35**
	SD	**1495.5**	**0.37**	**0.27**	**0.55**

Group	Participant	Average energy intake ($kJ \cdot day^{-1}$)	Average protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Average fat intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Average CHO intake ($g \cdot kg^{-1} \cdot day^{-1}$)
HIT+RT	1	10286	1.3	1.5	2.1
HIT+RT	2	9145.8	2.0	1.7	3.1
HIT+RT	3	10149	1.5	1.7	3.1
HIT+RT	4	7379.5	1.1	1.2	2.1
HIT+RT	5	9903.6	1.4	1.6	3.2
HIT+RT	6	9699.5	1.0	1.9	3.4
HIT+RT	7	5925.4	0.9	0.6	1.9
HIT+RT	8	7624.9	1.1	1.2	2.0
	Mean	**8764.2**	**1.29**	**1.42**	**2.61**
	SD	**1595.8**	**0.34**	**0.43**	**0.63**

Group	Participant	Average energy intake ($kJ \cdot day^{-1}$)	Average protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Average fat intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Average CHO intake ($g \cdot kg^{-1} \cdot day^{-1}$)
MICT+RT	1	6548.4	1.02	1.04	2.07
MICT+RT	2	9152.2	1.52	1.22	3.08
MICT+RT	3	6654.7	0.76	0.76	2.58
MICT+RT	4	7308.2	1.15	0.76	2.72
MICT+RT	5	9853.7	1.26	1.16	3.71
MICT+RT	6	8930.9	1.41	1.18	3.04
MICT+RT	7	6876	0.87	0.80	2.62
	Mean	**7903.4**	**1.14**	**0.99**	**2.83**
	SD	**1367.7**	**0.28**	**0.21**	**0.51**

Study 2: Western blotting data

p-mTOR Ser2448

Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	0.5	0.7	0.6	2.0
RT only	2	0.6	2.2	3.5	3.1
RT only	3	2.3	0.5	4.0	6.2
RT only	4	0.6	0.2	0.8	2.5
RT only	5	2.8	4.3	3.6	1.1
RT only	6	0.3	0.3	0.3	0.6
RT only	7	0.5	0.5	1.1	0.8
RT only	8	0.3	0.5	2.9	1.6
	Mean	**1.0**	**1.2**	**2.1**	**2.2**
	SD	**1.0**	**1.4**	**1.5**	**1.8**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	1.0	0.8	3.7	2.3
HIT+RT	2	1.1	1.7	1.8	2.0
HIT+RT	3	1.5	1.0	1.2	1.2
HIT+RT	4	1.2	1.5	1.5	1.4
HIT+RT	5	1.4	1.3	1.9	1.4
HIT+RT	6	0.6	0.4	1.1	1.1
HIT+RT	7	0.6	1.2	0.6	2.2
HIT+RT	8	0.6	0.8	0.8	3.4
	Mean	**1.0**	**1.1**	**1.6**	**1.9**
	SD	**0.4**	**0.4**	**1.0**	**0.8**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	1.9	0.5	0.4	0.6
MICT+RT	2	0.4	0.8	0.8	1.0
MICT+RT	3	0.4	0.3	0.3	0.2
MICT+RT	4	0.3	0.7	1.9	1.3
MICT+RT	5	1.8	1.0	1.0	2.2
MICT+RT	6	1.0	0.3	4.9	0.5
MICT+RT	7	1.1	1.4	1.6	2.7
	Mean	**1.0**	**0.7**	**1.6**	**1.2**
	SD	**0.7**	**0.4**	**1.6**	**0.9**

p-p70S6K1 Thr389
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.5	2.8	4.1	7.5
RT only	2	0.5	1.0	2.3	3.1
RT only	3	0.7	1.6	1.7	4.5
RT only	4	1.0	0.9	1.8	2.6
RT only	5	1.0	3.6	3.7	0.6
RT only	6	0.7	0.6	1.8	5.4
RT only	7	1.8	0.5	2.4	1.7
RT only	8	0.8	2.5	2.3	3.1
	Mean	**1.0**	**1.7**	**2.5**	**3.6**
	SD	**0.4**	**1.2**	**0.9**	**2.2**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.4	2.1	1.4	2.1
HIT+RT	2	1.8	1.5	1.0	4.9
HIT+RT	3	0.4	0.9	1.3	1.2
HIT+RT	4	1.2	1.4	1.7	0.7
HIT+RT	5	0.4	1.1	4.2	1.6
HIT+RT	6	1.4	2.7	4.8	2.5
HIT+RT	7	0.5	1.7	1.1	1.9
HIT+RT	8	1.9	1.8	1.1	1.4
	Mean	**1.0**	**1.7**	**2.1**	**2.0**
	SD	**0.7**	**0.6**	**1.5**	**1.3**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	1.1	2.3	1.1	2.0
MICT+RT	2	1.4	1.1	1.3	1.5
MICT+RT	3	0.9	0.7	1.5	1.5
MICT+RT	4	1.5	2.2	1.5	1.2
MICT+RT	5	0.8	0.6	2.0	0.8
MICT+RT	6	0.4	1.1	1.7	1.0
MICT+RT	7	0.8	1.3	2.0	1.3
	Mean	**1.0**	**1.3**	**1.6**	**1.3**
	SD	**0.4**	**0.7**	**0.3**	**0.4**

p-rps6 Ser235/236
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	0.3	0.6	14.9	9.3
RT only	2	0.6	0.9	2.3	16.4
RT only	3	0.4	0.9	8.0	27.6
RT only	4	2.0	4.3	13.3	8.1
RT only	5	1.3	0.1	4.2	6.5
RT only	6	0.4	0.2	0.6	1.1
RT only	7	0.2	0.2	1.8	0.5
RT only	8	2.8	9.4	91.3	113.8
	Mean	**1.0**	**2.1**	**17.1**	**22.9**
	SD	**0.9**	**3.2**	**30.5**	**37.8**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	1.1	2.2	60.3	5.6
HIT+RT	2	2.7	2.7	2.7	9.7
HIT+RT	3	0.8	0.8	18.9	24.1
HIT+RT	4	1.5	0.8	5.7	1.6
HIT+RT	5	1.0	2.5	26.2	7.5
HIT+RT	6	0.7	0.4	1.6	0.7
HIT+RT	7	0.1	0.2	0.2	0.3
HIT+RT	8	0.2	0.2	1.0	2.2
	Mean	**1.0**	**1.2**	**14.6**	**6.5**
	SD	**0.8**	**1.1**	**20.8**	**7.9**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.3	0.4	1.1	1.8
MICT+RT	2	0.2	0.4	4.9	2.6
MICT+RT	3	1.4	0.8	4.0	5.8
MICT+RT	4	1.8	1.2	10.8	1.7
MICT+RT	5	1.5	1.1	17.3	3.1
MICT+RT	6	0.7	1.0	12.1	2.7
MICT+RT	7	1.1	1.5	5.4	0.8
	Mean	**1.0**	**0.9**	**7.9**	**2.7**
	SD	**0.6**	**0.4**	**5.6**	**1.6**

p-GSK-3β Ser9
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	2.6	1.6	1.8	2.7
RT only	2	0.7	1.5	1.4	2.3
RT only	3	1.8	1.0	0.3	0.3
RT only	4	0.9	0.9	1.9	2.0
RT only	5	0.3	0.8	1.2	0.9
RT only	6	0.7	0.6	0.6	0.8
RT only	7	0.6	0.5	0.8	0.7
RT only	8	0.4	2.0	1.9	2.1
	Mean	**1.0**	**1.1**	**1.2**	**1.5**
	SD	**0.8**	**0.5**	**0.6**	**0.9**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.5	1.1	1.8	1.6
HIT+RT	2	1.1	1.4	1.6	3.0
HIT+RT	3	0.9	0.9	1.1	1.2
HIT+RT	4	1.0	0.9	1.0	0.9
HIT+RT	5	1.2	1.2	1.9	1.3
HIT+RT	6	0.9	0.9	1.5	1.4
HIT+RT	7	1.3	1.4	1.3	1.4
HIT+RT	8	1.2	1.9	1.5	1.5
	Mean	**1.0**	**1.2**	**1.5**	**1.5**
	SD	**0.3**	**0.3**	**0.3**	**0.6**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.9	1.3	1.5	1.6
MICT+RT	2	1.4	1.8	1.9	1.9
MICT+RT	3	0.8	0.9	0.9	1.4
MICT+RT	4	1.5	1.6	1.4	1.5
MICT+RT	5	0.9	1.1	1.5	0.9
MICT+RT	6	0.7	0.7	1.0	0.8
MICT+RT	7	0.7	1.2	1.4	1.0
	Mean	**1.0**	**1.2**	**1.4**	**1.3**
	SD	**0.3**	**0.4**	**0.3**	**0.4**

p-TIF-1A Ser649
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.3	3.9	7.2	23.3
RT only	2	1.5	1.4	2.0	14.3
RT only	3	0.8	1.8	3.1	8.7
RT only	4	2.0	1.3	2.6	2.5
RT only	5	0.6	2.0	5.5	0.6
RT only	6	0.4	0.4	0.9	3.5
RT only	7	1.0	0.2	1.5	0.8
RT only	8	0.4	1.5	2.5	4.6
	Mean	**1.0**	**1.6**	**3.1**	**7.3**
	SD	**0.6**	**1.1**	**2.1**	**7.9**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.2	1.8	3.6	2.1
HIT+RT	2	1.4	1.7	1.1	7.7
HIT+RT	3	0.6	2.9	3.3	3.0
HIT+RT	4	1.3	1.3	2.2	1.1
HIT+RT	5	0.4	0.6	3.1	0.6
HIT+RT	6	0.8	2.8	3.9	2.1
HIT+RT	7	0.8	3.4	1.9	9.6
HIT+RT	8	2.6	1.6	1.5	7.9
	Mean	**1.0**	**2.0**	**2.6**	**4.2**
	SD	**0.8**	**1.0**	**1.0**	**3.5**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.6	1.1	0.7	1.4
MICT+RT	2	0.9	0.8	1.2	0.3
MICT+RT	3	1.5	1.5	3.9	2.8
MICT+RT	4	2.6	2.3	1.3	1.1
MICT+RT	5	0.6	0.4	2.5	0.7
MICT+RT	6	0.4	1.3	1.0	0.9
MICT+RT	7	0.5	1.1	1.1	0.8
	Mean	**1.0**	**1.2**	**1.7**	**1.1**
	SD	**0.8**	**0.6**	**1.1**	**0.8**

p-UBF Ser388
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	0.8	0.9	1.6	3.2
RT only	2	0.5	0.9	0.9	2.5
RT only	3	0.6	0.6	1.7	2.1
RT only	4	0.4	0.6	1.2	1.1
RT only	5	0.7	1.2	1.9	1.1
RT only	6	1.8	1.0	1.8	2.9
RT only	7	2.2	0.6	2.5	1.3
RT only	8	1.0	0.9	0.8	1.7
	Mean	**1.0**	**0.8**	**1.6**	**2.0**
	SD	**0.6**	**0.2**	**0.5**	**0.8**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.7	1.4	1.9	1.5
HIT+RT	2	1.2	1.1	1.4	2.4
HIT+RT	3	0.9	1.0	1.3	1.2
HIT+RT	4	1.0	0.9	1.1	0.8
HIT+RT	5	0.7	0.8	1.2	0.7
HIT+RT	6	0.9	1.5	2.1	1.8
HIT+RT	7	0.8	1.2	1.2	1.6
HIT+RT	8	1.7	1.3	1.1	1.3
	Mean	**1.0**	**1.2**	**1.4**	**1.4**
	SD	**0.3**	**0.3**	**0.4**	**0.5**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	1.4	2.9	1.8	1.8
MICT+RT	2	1.9	1.5	1.5	1.7
MICT+RT	3	1.1	1.1	1.7	0.9
MICT+RT	4	0.9	0.8	1.2	0.8
MICT+RT	5	0.6	0.8	1.8	0.8
MICT+RT	6	0.7	0.9	0.7	0.6
MICT+RT	7	0.4	0.8	0.8	0.5
	Mean	**1.0**	**1.3**	**1.3**	**1.0**
	SD	**0.5**	**0.8**	**0.5**	**0.5**

p-4E-BP1 Thr 37/46
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.2	1.0	0.8	1.0
RT only	2	1.2	1.2	1.6	0.7
RT only	3	1.7	1.2	1.1	1.4
RT only	4	1.3	1.3	1.3	0.7
RT only	5	1.4	1.2	0.7	1.1
RT only	6	0.3	0.6	0.4	0.7
RT only	7	0.4	1.1	1.0	1.4
RT only	8	0.5	2.0	3.0	1.6
	Mean	**1.0**	**1.2**	**1.2**	**1.1**
	SD	**0.5**	**0.4**	**0.8**	**0.3**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.6	0.8	0.6	0.9
HIT+RT	2	1.1	1.1	0.9	0.6
HIT+RT	3	0.7	0.9	1.3	1.2
HIT+RT	4	1.8	1.2	0.8	1.3
HIT+RT	5	0.8	0.8	0.7	1.0
HIT+RT	6	1.5	0.8	1.5	1.6
HIT+RT	7	0.7	0.5	0.6	0.8
HIT+RT	8	0.6	1.1	0.9	1.2
	Mean	**1.0**	**0.9**	**0.9**	**1.1**
	SD	**0.4**	**0.2**	**0.3**	**0.3**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.7	1.5	1.2	0.7
MICT+RT	2	0.7	1.2	2.1	2.0
MICT+RT	3	1.0	0.6	0.8	1.2
MICT+RT	4	1.3	1.3	1.7	2.0
MICT+RT	5	1.4	1.1	1.2	1.4
MICT+RT	6	1.0	1.7	1.4	1.7
MICT+RT	7	1.0	1.1	0.9	2.6
	Mean	**1.0**	**1.2**	**1.3**	**1.6**
	SD	**0.3**	**0.4**	**0.5**	**0.6**

p-AMPK Thr172
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	3.2	4.5	7.6	0.0
RT only	2	0.6	1.0	1.3	3.8
RT only	3	0.4	1.6	3.4	4.6
RT only	4	0.6	0.4	0.8	1.0
RT only	5	0.4	1.8	3.5	0.4
RT only	6	0.9	0.5	1.1	4.3
RT only	7	1.5	0.2	0.9	0.6
RT only	8	0.3	1.5	0.9	1.2
	Mean	**1.0**	**1.4**	**2.4**	**2.0**
	SD	**1.0**	**1.4**	**2.4**	**1.9**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	1.0	1.3	1.6	1.1
HIT+RT	2	0.5	0.2	2.2	0.2
HIT+RT	3	1.1	1.1	0.5	1.2
HIT+RT	4	1.1	1.0	0.9	0.6
HIT+RT	5	0.4	1.6	0.9	0.8
HIT+RT	6	2.8	2.4	3.0	1.6
HIT+RT	7	0.2	0.5	0.4	1.0
HIT+RT	8	0.8	0.3	0.3	0.4
	Mean	**1.0**	**1.1**	**1.2**	**0.9**
	SD	**0.8**	**0.7**	**0.9**	**0.4**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.7	1.2	0.9	1.1
MICT+RT	2	1.3	1.0	1.1	0.6
MICT+RT	3	2.1	2.6	3.0	1.8
MICT+RT	4	1.5	2.1	1.8	1.0
MICT+RT	5	0.4	0.4	1.2	0.4
MICT+RT	6	0.1	0.5	0.7	0.7
MICT+RT	7	0.8	1.4	2.2	1.2
	Mean	**1.0**	**1.3**	**1.5**	**1.0**
	SD	**0.7**	**0.8**	**0.8**	**0.5**

p-ACC Ser79
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	0.2	0.3	0.1	0.1
RT only	2	1.1	2.2	1.2	0.8
RT only	3	2.5	2.7	1.1	0.8
RT only	4	1.0	1.2	1.4	1.6
RT only	5	1.3	0.6	0.3	0.5
RT only	6	0.4	0.6	0.5	0.4
RT only	7	1.0	3.7	2.3	1.7
RT only	8	0.4	1.4	1.5	0.7
	Mean	**1.0**	**1.6**	**1.0**	**0.8**
	SD	**0.7**	**1.2**	**0.7**	**0.6**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.1	0.3	1.2	1.0
HIT+RT	2	2.9	2.7	2.6	3.0
HIT+RT	3	0.4	0.4	0.3	1.7
HIT+RT	4	1.2	0.9	1.1	1.3
HIT+RT	5	1.5	1.4	1.0	1.7
HIT+RT	6	1.0	0.8	0.9	1.6
HIT+RT	7	0.6	0.6	0.8	0.3
HIT+RT	8	0.4	0.8	1.5	0.7
	Mean	**1.0**	**1.0**	**1.2**	**1.4**
	SD	**0.9**	**0.8**	**0.7**	**0.8**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	2.0	2.5	1.5	1.0
MICT+RT	2	1.5	1.6	0.4	0.8
MICT+RT	3	0.2	0.2	0.2	0.7
MICT+RT	4	1.6	1.0	0.7	0.7
MICT+RT	5	0.3	0.7	0.4	0.6
MICT+RT	6	0.5	0.5	0.4	0.5
MICT+RT	7	0.9	0.9	0.3	0.4
	Mean	**1.0**	**1.1**	**0.6**	**0.7**
	SD	**0.7**	**0.8**	**0.5**	**0.2**

p-eEF2 Thr56
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.8	1.1	1.2	1.2
RT only	2	0.4	0.5	0.2	2.0
RT only	3	0.3	0.8	0.9	0.6
RT only	4	0.7	0.6	0.4	0.2
RT only	5	0.3	0.4	0.3	0.2
RT only	6	3.6	1.3	0.4	0.5
RT only	7	0.6	0.6	0.5	0.5
RT only	8	0.4	2.0	2.1	1.9
	Mean	**1.0**	**0.9**	**0.8**	**0.9**
	SD	**1.1**	**0.6**	**0.6**	**0.7**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	1.3	1.5	0.5	1.1
HIT+RT	2	0.6	0.5	0.4	0.9
HIT+RT	3	1.4	1.4	0.8	0.7
HIT+RT	4	1.2	1.3	0.5	0.4
HIT+RT	5	1.6	1.1	0.7	0.6
HIT+RT	6	0.3	0.6	0.4	0.6
HIT+RT	7	1.2	1.2	0.5	0.9
HIT+RT	8	0.4	0.6	0.4	0.2
	Mean	**1.0**	**1.0**	**0.5**	**0.7**
	SD	**0.5**	**0.4**	**0.2**	**0.3**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.7	0.9	0.7	0.6
MICT+RT	2	2.1	2.3	1.0	1.5
MICT+RT	3	1.5	1.1	0.6	1.0
MICT+RT	4	0.9	1.1	0.6	0.5
MICT+RT	5	0.6	0.5	0.4	0.4
MICT+RT	6	0.7	0.8	0.2	0.4
MICT+RT	7	0.5	0.8	1.1	0.5
	Mean	**1.0**	**1.1**	**0.7**	**0.7**
	SD	**0.6**	**0.6**	**0.3**	**0.4**

Total TIF-1A
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	0.8	0.7	0.6	0.4
RT only	2	0.8	1.1	0.8	0.2
RT only	3	1.1	0.9	1.0	0.4
RT only	4	0.5	0.6	0.7	0.7
RT only	5	1.0	0.8	0.4	0.6
RT only	6	1.3	1.4	1.2	0.9
RT only	7	1.4	1.6	1.0	1.1
RT only	8	1.1	0.8	0.3	0.5
	Mean	**1.0**	**1.0**	**0.7**	**0.6**
	SD	**0.3**	**0.3**	**0.3**	**0.3**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	1.2	0.9	0.5	0.9
HIT+RT	2	1.0	0.8	1.2	1.0
HIT+RT	3	0.9	0.6	0.6	0.5
HIT+RT	4	1.0	0.9	0.7	0.8
HIT+RT	5	1.3	1.2	0.9	0.9
HIT+RT	6	1.1	0.9	1.1	1.1
HIT+RT	7	0.7	0.6	0.7	0.2
HIT+RT	8	0.7	0.9	0.6	0.1
	Mean	**1.0**	**0.8**	**0.8**	**0.7**
	SD	**0.2**	**0.2**	**0.2**	**0.4**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	1.4	1.8	1.5	0.9
MICT+RT	2	1.5	1.0	0.6	1.5
MICT+RT	3	0.7	0.8	0.5	0.6
MICT+RT	4	0.5	0.8	0.9	0.7
MICT+RT	5	1.0	1.0	0.7	1.1
MICT+RT	6	0.8	0.5	0.0	0.4
MICT+RT	7	1.2	0.8	0.4	0.7
	Mean	**1.0**	**0.9**	**0.7**	**0.8**
	SD	**0.4**	**0.4**	**0.5**	**0.4**

Total Cyclin D1
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.5	1.7	1.3	0.9
RT only	2	1.8	1.5	1.5	0.9
RT only	3	0.9	1.1	1.0	0.9
RT only	4	1.1	1.1	0.7	0.8
RT only	5	1.1	1.2	1.2	1.4
RT only	6	0.2	0.3	0.2	0.2
RT only	7	0.2	0.2	0.2	0.2
RT only	8				
	Mean	**1.0**	**1.0**	**0.9**	**0.7**
	SD	**0.6**	**0.6**	**0.5**	**0.4**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.7	0.6	0.3	0.7
HIT+RT	2	0.5	0.9	0.4	0.8
HIT+RT	3	1.5	1.2	1.2	1.0
HIT+RT	4	0.8	0.7	0.5	0.7
HIT+RT	5	0.7	0.8	0.4	0.5
HIT+RT	6	1.2	1.1	0.9	0.8
HIT+RT	7	1.9	0.8	0.7	0.9
HIT+RT	8	0.7	0.7	0.4	0.6
	Mean	**1.0**	**0.8**	**0.6**	**0.7**
	SD	**0.5**	**0.2**	**0.3**	**0.2**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.2	0.3	0.4	0.6
MICT+RT	2	0.4	0.4	0.3	0.5
MICT+RT	3	1.4	1.1	0.5	0.7
MICT+RT	4	1.4	1.1	1.3	1.2
MICT+RT	5	0.9	0.7	0.5	0.5
MICT+RT	6	1.3	1.0	0.5	1.1
MICT+RT	7	1.4	0.8	1.1	1.7
	Mean	**1.0**	**0.8**	**0.7**	**0.9**
	SD	**0.5**	**0.3**	**0.4**	**0.4**

Total mTOR
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.1	0.9	1.5	0.8
RT only	2	1.0	1.2	1.0	2.0
RT only	3	1.0	0.8	1.0	0.8
RT only	4	0.7	0.9	1.2	1.8
RT only	5	1.9	1.1	1.7	1.9
RT only	6	0.5	1.0	0.8	0.9
RT only	7	0.8	1.1	1.7	2.2
RT only	8	1.0	0.9	0.6	0.6
	Mean	**1.0**	**1.0**	**1.2**	**1.4**
	SD	**0.4**	**0.1**	**0.4**	**0.7**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.9	1.3	0.9	1.0
HIT+RT	2	1.5	1.3	1.5	1.3
HIT+RT	3	0.8	1.1	1.3	1.3
HIT+RT	4	1.2	0.9	1.1	1.1
HIT+RT	5	1.2	1.5	1.1	1.4
HIT+RT	6	0.6	0.8	0.6	0.6
HIT+RT	7	0.8	0.7	0.7	0.7
HIT+RT	8	1.0	0.9	0.5	0.3
	Mean	**1.0**	**1.1**	**1.0**	**1.0**
	SD	**0.3**	**0.3**	**0.3**	**0.4**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.8	1.2	1.2	1.1
MICT+RT	2	1.1	1.8	1.1	1.6
MICT+RT	3	0.8	0.9	1.4	0.9
MICT+RT	4	0.8	0.5	0.6	1.0
MICT+RT	5	1.1	1.3	1.3	1.2
MICT+RT	6	1.5	2.1	1.0	1.1
MICT+RT	7	0.9	1.7	1.7	0.8
	Mean	**1.0**	**1.4**	**1.2**	**1.1**
	SD	**0.3**	**0.5**	**0.4**	**0.3**

Total UBF
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.3	1.4	1.5	1.6
RT only	2	1.1	1.3	1.0	1.1
RT only	3	1.0	1.1	1.2	1.1
RT only	4	1.0	0.9	0.9	1.1
RT only	5	1.0	1.1	1.1	0.9
RT only	6	0.9	0.8	0.9	1.1
RT only	7	0.7	0.7	0.7	0.8
RT only	8	0.8	0.9	0.5	0.5
	Mean	**1.0**	**1.0**	**1.0**	**1.0**
	SD	**0.2**	**0.2**	**0.3**	**0.3**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.8	1.3	1.0	0.9
HIT+RT	2	0.9	0.9	1.0	1.7
HIT+RT	3	0.8	0.6	0.8	0.8
HIT+RT	4	0.8	0.7	0.8	0.7
HIT+RT	5	0.7	0.8	1.2	0.9
HIT+RT	6	1.7	2.0	1.9	1.7
HIT+RT	7	1.1	1.3	1.5	1.4
HIT+RT	8	1.1	1.4	1.2	1.5
	Mean	**1.0**	**1.1**	**1.2**	**1.2**
	SD	**0.3**	**0.5**	**0.4**	**0.4**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.9	1.4	1.2	1.3
MICT+RT	2	0.7	0.8	0.8	1.3
MICT+RT	3	1.3	1.3	1.5	1.3
MICT+RT	4	0.8	1.0	1.0	0.9
MICT+RT	5	0.9	1.0	1.2	1.2
MICT+RT	6	1.2	1.3	0.7	1.1
MICT+RT	7	1.1	1.5	1.3	1.3
	Mean	**1.0**	**1.2**	**1.1**	**1.2**
	SD	**0.2**	**0.3**	**0.3**	**0.1**

Total rps6
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	0.7	0.9	1.0	0.9
RT only	2	0.9	0.9	0.8	0.8
RT only	3	0.8	0.8	1.2	0.7
RT only	4	0.8	0.7	0.9	0.9
RT only	5	0.9	1.0	0.9	1.0
RT only	6	0.7	1.0	1.8	1.9
RT only	7	1.6	1.4	1.2	1.8
RT only	8	1.5	0.9	1.3	1.5
	Mean	**1.0**	**1.0**	**1.1**	**1.2**
	SD	**0.3**	**0.2**	**0.3**	**0.5**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.1	0.2	0.2	0.3
HIT+RT	2	0.4	0.4	0.3	0.4
HIT+RT	3	0.2	0.3	0.3	0.4
HIT+RT	4	0.3	0.3	0.3	0.3
HIT+RT	5	0.3	0.3	0.3	0.3
HIT+RT	6	2.5	2.3	1.8	2.1
HIT+RT	7	2.0	2.3	2.3	2.4
HIT+RT	8	2.2	2.2	2.7	3.3
	Mean	**1.0**	**1.0**	**1.0**	**1.2**
	SD	**1.0**	**1.0**	**1.1**	**1.2**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	2.8	2.5	3.5	4.7
MICT+RT	2	2.6	5.6	4.4	5.5
MICT+RT	3	0.3	0.4	0.4	0.3
MICT+RT	4	0.3	0.3	0.5	0.5
MICT+RT	5	0.3	0.3	0.4	0.4
MICT+RT	6	0.4	0.4	0.6	0.4
MICT+RT	7	0.3	0.3	0.3	0.3
	Mean	**1.0**	**1.4**	**1.4**	**1.7**
	SD	**1.2**	**2.0**	**1.7**	**2.3**

Total 4E-BP1
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	0.5	0.8	0.7	1.0
RT only	2	1.4	1.7	1.3	1.5
RT only	3	0.6	0.7	0.9	1.1
RT only	4	0.9	0.9	0.9	0.8
RT only	5	1.3	1.1	1.3	1.3
RT only	6	1.2	0.9	1.3	1.1
RT only	7	1.3	1.1	1.1	0.8
RT only	8	0.8	0.5	0.1	0.0
	Mean	**1.0**	**1.0**	**0.9**	**0.9**
	SD	**0.3**	**0.4**	**0.4**	**0.4**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	0.8	1.2	1.3	1.3
HIT+RT	2	1.4	1.4	1.3	1.1
HIT+RT	3	0.8	0.9	0.9	0.9
HIT+RT	4	0.5	0.7	0.7	0.8
HIT+RT	5	0.9	0.8	0.8	0.8
HIT+RT	6	0.6	0.9	0.9	1.1
HIT+RT	7	0.9	1.4	1.1	1.1
HIT+RT	8	1.9	1.5	1.4	1.3
	Mean	**1.0**	**1.1**	**1.1**	**1.0**
	SD	**0.4**	**0.3**	**0.3**	**0.2**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	2.1	2.3	1.8	1.6
MICT+RT	2	1.1	1.0	0.8	1.0
MICT+RT	3	0.8	0.8	0.8	0.8
MICT+RT	4	1.0	1.0	1.0	1.1
MICT+RT	5	0.8	0.8	1.0	0.8
MICT+RT	6	0.6	0.8	1.6	0.7
MICT+RT	7	0.5	0.7	0.5	0.5
	Mean	**1.0**	**1.1**	**1.1**	**0.9**
	SD	**0.5**	**0.6**	**0.5**	**0.4**

Total AMPK
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+1 h	+3 h
RT only	1	1.1	1.0	1.0	0.8
RT only	2	1.4	1.5	1.3	1.3
RT only	3	1.0	1.0	1.2	1.0
RT only	4	0.9	0.9	1.0	1.2
RT only	5	1.0	1.2	1.0	1.1
RT only	6	1.1	1.2	1.0	0.9
RT only	7	0.7	0.9	0.8	0.8
RT only	8	0.8	0.9	1.1	1.0
	Mean	**1.0**	**1.1**	**1.0**	**1.0**
	SD	**0.2**	**0.2**	**0.2**	**0.2**

Group	Participant	PRE	POST	+1 h	+3 h
HIT+RT	1	1.0	1.0	1.4	0.8
HIT+RT	2	1.3	1.5	1.9	1.4
HIT+RT	3	0.7	1.5	1.3	1.2
HIT+RT	4	1.1	1.1	1.3	1.0
HIT+RT	5	1.1	1.1	1.0	0.6
HIT+RT	6	0.5	1.2	1.4	1.2
HIT+RT	7	0.9	1.5	1.2	1.3
HIT+RT	8	1.3	1.4	1.4	1.2
	Mean	**1.0**	**1.3**	**1.4**	**1.1**
	SD	**0.3**	**0.2**	**0.3**	**0.3**

Group	Participant	PRE	POST	+1 h	+3 h
MICT+RT	1	0.9	1.6	1.0	1.4
MICT+RT	2	1.3	1.0	1.1	1.2
MICT+RT	3	1.1	1.1	1.2	1.2
MICT+RT	4	1.0	1.1	1.1	1.0
MICT+RT	5	1.0	1.3	1.2	1.2
MICT+RT	6	1.0	1.0	0.8	0.8
MICT+RT	7	0.6	0.8	0.7	0.6
	Mean	**1.0**	**1.1**	**1.0**	**1.1**
	SD	**0.2**	**0.3**	**0.2**	**0.3**

Study 2: qPCR data

MuRF-1 mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	0.7	0.4	0.8
RT only	2	1.5	1.2	0.4
RT only	3	0.8	0.7	0.8
RT only	4	0.8	1.5	0.7
RT only	5	1.1	0.7	1.5
RT only	6	1.5	0.7	1.0
RT only	7	0.8	0.9	0.9
RT only	8	0.9	0.5	1.2
	Mean	**1.0**	**0.8**	**0.9**
	SD	**0.3**	**0.4**	**0.3**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.5	0.8	9.4
HIT+RT	2	1.1	0.9	2.8
HIT+RT	3	1.0	0.9	2.9
HIT+RT	4	0.7	0.6	2.7
HIT+RT	5	1.6	1.1	1.3
HIT+RT	6	0.4	0.9	1.3
HIT+RT	7	1.1	0.8	2.1
HIT+RT	8	0.7	0.5	1.4
	Mean	**1.0**	**0.8**	**3.0**
	SD	**0.4**	**0.2**	**2.7**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.2	1.3	2.2
MICT+RT	2	1.5	1.2	0.5
MICT+RT	3	1.6	1.0	0.7
MICT+RT	4	1.0	1.0	1.5
MICT+RT	5	0.5	0.4	1.6
MICT+RT	6	0.5	1.0	3.6
MICT+RT	7	0.7	1.1	0.5
	Mean	**1.0**	**1.0**	**1.5**
	SD	**0.5**	**0.3**	**1.1**

Atrogin-1 mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	0.6	0.5	0.4
RT only	2	1.4	0.9	0.3
RT only	3	0.9	0.9	0.6
RT only	4	0.5	1.7	0.3
RT only	5	1.5	0.8	0.7
RT only	6	0.9	0.7	0.5
RT only	7	0.9	0.9	0.5
RT only	8	1.2	0.5	0.4
	Mean	**1.0**	**0.9**	**0.5**
	SD	**0.4**	**0.4**	**0.1**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.5	1.0	1.4
HIT+RT	2	0.6	0.4	0.7
HIT+RT	3	1.1	1.0	0.9
HIT+RT	4	1.2	1.0	1.5
HIT+RT	5	1.1	1.2	1.0
HIT+RT	6	0.5	0.7	0.7
HIT+RT	7	1.4	0.6	0.8
HIT+RT	8	0.5	0.7	0.3
	Mean	**1.0**	**0.8**	**0.9**
	SD	**0.4**	**0.3**	**0.4**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.7	0.5	1.0
MICT+RT	2	1.3	0.6	0.6
MICT+RT	3	1.2	0.8	0.6
MICT+RT	4	1.1	1.4	0.8
MICT+RT	5	0.5	0.5	0.7
MICT+RT	6	0.6	0.9	1.6
MICT+RT	7	0.6	1.4	0.9
	Mean	**1.0**	**0.9**	**0.9**
	SD	**0.5**	**0.4**	**0.3**

PGC-1α mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	0.5	0.6	1.1
RT only	2	1.3	0.9	0.4
RT only	3	0.8	0.5	0.9
RT only	4	1.1	1.2	1.0
RT only	5	1.8	1.0	1.2
RT only	6	0.9	0.5	1.3
RT only	7	0.5	0.5	0.6
RT only	8	1.0	0.6	0.7
	Mean	**1.0**	**0.7**	**0.9**
	SD	**0.4**	**0.3**	**0.3**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	0.9	0.8	11.2
HIT+RT	2	1.0	1.3	5.6
HIT+RT	3	1.2	0.7	12.1
HIT+RT	4	1.5	0.9	10.9
HIT+RT	5	1.0	0.9	6.8
HIT+RT	6	0.6	0.7	7.2
HIT+RT	7	1.1	0.8	4.3
HIT+RT	8	0.7	0.9	8.7
	Mean	**1.0**	**0.9**	**8.4**
	SD	**0.3**	**0.2**	**2.8**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	0.6	0.4	3.6
MICT+RT	2	0.4	0.2	2.5
MICT+RT	3	0.5	0.4	0.9
MICT+RT	4	0.7	0.4	3.8
MICT+RT	5	4.1	0.3	2.3
MICT+RT	6	0.2	0.3	5.0
MICT+RT	7	0.5	0.4	1.1
	Mean	**1.0**	**0.4**	**2.7**
	SD	**1.4**	**0.1**	**1.5**

TIF-1A mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	0.9	1.1	1.5
RT only	2	1.1	0.9	1.2
RT only	3	1.6	1.1	1.3
RT only	4	0.8	0.8	1.3
RT only	5	1.2	1.1	1.2
RT only	6	0.8	1.0	1.3
RT only	7	0.7	0.5	0.7
RT only	8	0.9	0.9	0.9
	Mean	**1.0**	**0.9**	**1.2**
	SD	**0.3**	**0.2**	**0.2**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	0.8	0.9	0.5
HIT+RT	2	0.9	0.7	0.5
HIT+RT	3	1.4	1.3	2.1
HIT+RT	4	1.4	1.2	1.2
HIT+RT	5	1.0	0.8	1.1
HIT+RT	6	1.1	0.7	0.9
HIT+RT	7	0.7	0.6	0.8
HIT+RT	8	0.8	0.9	1.5
	Mean	**1.0**	**0.9**	**1.1**
	SD	**0.3**	**0.2**	**0.5**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.3	1.0	1.5
MICT+RT	2	0.9	0.8	1.7
MICT+RT	3	1.0	1.0	1.2
MICT+RT	4	1.2	1.2	1.4
MICT+RT	5	0.7	0.7	0.8
MICT+RT	6	0.9	0.9	1.0
MICT+RT	7			
	Mean	**1.0**	**0.9**	**1.2**
	SD	**0.2**	**0.2**	**0.3**

UBF mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.1	0.9	0.9
RT only	2	1.1	0.9	0.5
RT only	3	0.9	0.7	1.0
RT only	4	1.0	1.2	0.4
RT only	5	1.2	1.0	1.0
RT only	6	1.0	0.7	0.6
RT only	7	0.7	1.0	0.6
RT only	8	0.9	0.7	0.7
	Mean	**1.0**	**0.9**	**0.7**
	SD	**0.2**	**0.2**	**0.2**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.3	1.2	1.7
HIT+RT	2	0.8	1.1	0.9
HIT+RT	3	1.3	1.1	1.5
HIT+RT	4	1.0	0.9	0.9
HIT+RT	5	1.1	1.3	1.0
HIT+RT	6	0.8	1.0	0.6
HIT+RT	7	1.2	1.0	0.7
HIT+RT	8	0.5	0.8	0.7
	Mean	**1.0**	**1.0**	**1.0**
	SD	**0.3**	**0.2**	**0.4**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.0	1.1	0.8
MICT+RT	2	1.2	1.3	0.9
MICT+RT	3	1.3	1.0	0.7
MICT+RT	4	1.0	1.0	1.2
MICT+RT	5	0.8	0.7	0.8
MICT+RT	6	0.8	1.1	1.4
MICT+RT	7	0.9	1.2	0.7
	Mean	**1.0**	**1.0**	**0.9**
	SD	**0.2**	**0.2**	**0.2**

Cyclin D1 mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	2.0	1.2	2.7
RT only	2	1.7	1.8	0.9
RT only	3	1.1	1.3	1.1
RT only	4	0.6	1.0	1.5
RT only	5	1.2	0.9	1.4
RT only	6	0.6	0.4	0.4
RT only	7	0.0	0.6	0.9
RT only	8	0.7	0.9	0.6
	Mean	**1.0**	**1.0**	**1.2**
	SD	**0.6**	**0.4**	**0.7**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.6	4.4	10.0
HIT+RT	2	1.6	3.5	1.5
HIT+RT	3	0.8	1.6	2.4
HIT+RT	4	0.4	0.7	1.2
HIT+RT	5	0.7	1.0	1.4
HIT+RT	6	1.0	2.0	1.3
HIT+RT	7	0.7	2.8	3.2
HIT+RT	8	1.1	1.4	1.2
	Mean	**1.0**	**2.2**	**2.8**
	SD	**0.4**	**1.3**	**3.0**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.2	2.0	1.6
MICT+RT	2	1.4	2.6	2.0
MICT+RT	3	1.7	0.8	0.8
MICT+RT	4	0.9	1.6	2.8
MICT+RT	5	1.0	0.5	0.9
MICT+RT	6	0.4	0.8	1.2
MICT+RT	7	0.4	1.2	0.6
	Mean	**1.0**	**1.4**	**1.4**
	SD	**0.5**	**0.8**	**0.8**

POLR1B mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.0	0.8	1.2
RT only	2	1.0	0.9	0.8
RT only	3	1.0	0.7	1.4
RT only	4	0.9	1.2	0.6
RT only	5	1.7	0.9	1.1
RT only	6	0.8	0.5	0.6
RT only	7	0.7	0.4	0.4
RT only	8	1.0	0.6	0.5
	Mean	**1.0**	**0.8**	**0.8**
	SD	**0.3**	**0.3**	**0.4**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.4	1.4	2.9
HIT+RT	2	0.8	1.1	0.7
HIT+RT	3	0.9	1.0	2.4
HIT+RT	4	1.0	0.9	1.5
HIT+RT	5	1.2	1.1	1.6
HIT+RT	6	0.9	0.8	1.0
HIT+RT	7	0.9	0.9	1.2
HIT+RT	8	0.8	0.9	1.3
	Mean	**1.0**	**1.0**	**1.6**
	SD	**0.2**	**0.2**	**0.8**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.3	1.1	1.3
MICT+RT	2	1.2	1.2	1.9
MICT+RT	3	1.2	0.9	1.2
MICT+RT	4	1.3	1.3	1.8
MICT+RT	5	0.5	0.3	0.8
MICT+RT	6	0.8	0.8	2.0
MICT+RT	7	0.7	1.1	0.9
	Mean	**1.0**	**1.0**	**1.4**
	SD	**0.3**	**0.3**	**0.5**

Fox-O1 mRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.7	1.7	1.8
RT only	2	0.6	1.1	0.9
RT only	3	0.9	0.9	1.4
RT only	4	1.1	1.4	1.4
RT only	5	0.8	1.3	0.9
RT only	6	1.2	1.1	1.2
RT only	7	0.7	1.3	1.1
RT only	8	1.0	1.2	2.1
	Mean	**1.0**	**1.2**	**1.4**
	SD	**0.3**	**0.2**	**0.4**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.3	1.2	2.5
HIT+RT	2	1.2	1.6	5.2
HIT+RT	3	1.3	1.0	2.3
HIT+RT	4	1.2	1.1	1.7
HIT+RT	5	0.0	1.2	2.5
HIT+RT	6	1.0	0.5	1.4
HIT+RT	7	1.1	0.4	2.1
HIT+RT	8	0.9	1.4	3.9
	Mean	**1.0**	**1.0**	**2.7**
	SD	**0.4**	**0.4**	**1.2**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	0.7	0.8	2.3
MICT+RT	2	1.2	2.7	2.1
MICT+RT	3	1.1	1.9	1.0
MICT+RT	4	1.1	1.0	1.1
MICT+RT	5	0.3	0.7	2.9
MICT+RT	6	1.1	1.3	1.9
MICT+RT	7	1.4	1.2	1.4
	Mean	**1.0**	**1.4**	**1.8**
	SD	**0.4**	**0.7**	**0.7**

Total RNA (ng·mg^{-1} wet mass)

Group	Participant	PRE	POST	Δ PRE (ng·mg^{-1} wet mass)
RT only	1	860.9	709.6	-151.4
RT only	2	1228.4	947.2	-281.2
RT only	3	1144.7	660.5	-484.2
RT only	4	665.9	651.4	-14.5
RT only	5	1923.3	785.4	-1137.9
RT only	6	440.0	318.0	-122.0
RT only	7	379.8	371.5	-8.3
RT only	8	580.0	547.9	-32.1
	Mean	**902.9**	**623.9**	**-278.9**
	SD	**514.9**	**208.1**	**382.6**

Group	Participant	PRE	POST	Δ PRE (ng·mg^{-1} wet mass)
HIT+RT	1	451.0	605.7	154.7
HIT+RT	2	649.1	857.3	208.2
HIT+RT	3	259.4	607.9	348.5
HIT+RT	4	199.3	576.6	377.3
HIT+RT	5	535.0	744.3	209.3
HIT+RT	6	494.6	534.3	39.7
HIT+RT	7	606.6	685.9	79.3
HIT+RT	8	549.2	579.1	29.9
	Mean	**468.0**	**648.9**	**180.9**
	SD	**160.3**	**107.4**	**132.0**

Group	Participant	PRE	POST	Δ PRE (ng·mg^{-1} wet mass)
MICT+RT	1	701.4	681.2	-20.2
MICT+RT	2	490.7	1061.6	570.9
MICT+RT	3	639.2	629.1	-10.0
MICT+RT	4	558.1	759.4	201.4
MICT+RT	5	355.8	451.4	95.6
MICT+RT	6	360.4	463.5	103.1
MICT+RT	7	559.4	638.0	78.5
	Mean	**523.6**	**669.2**	**145.6**
	SD	**131.2**	**206.0**	**201.9**

45S pre-rRNA
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.3	1.1	2.3
RT only	2	1.9	0.6	0.4
RT only	3	1.2	0.4	0.5
RT only	4	0.3	0.8	0.4
RT only	5	1.5	0.9	1.3
RT only	6	0.5	0.4	0.7
RT only	7	0.4	0.3	0.4
RT only	8	0.7	0.4	0.4
	Mean	**1.0**	**0.6**	**0.8**
	SD	**0.6**	**0.3**	**0.7**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.8	2.3	6.9
HIT+RT	2	1.1	1.6	0.7
HIT+RT	3	0.8	1.0	6.0
HIT+RT	4	1.0	0.7	2.0
HIT+RT	5	0.9	0.9	1.7
HIT+RT	6	0.9	0.7	0.7
HIT+RT	7	0.9	0.8	1.1
HIT+RT	8	0.5	0.8	1.0
	Mean	**1.0**	**1.1**	**2.5**
	SD	**0.4**	**0.6**	**2.5**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.3	1.3	1.4
MICT+RT	2	1.5	2.7	2.6
MICT+RT	3	1.1	1.0	0.8
MICT+RT	4	1.2	1.3	4.6
MICT+RT	5	0.5	0.5	0.9
MICT+RT	6	0.7	1.0	2.1
MICT+RT	7	0.7	1.0	1.1
	Mean	**1.0**	**1.3**	**1.9**
	SD	**0.4**	**0.7**	**1.3**

5.8S rRNA (mature)
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.0	0.4	1.5
RT only	2	2.7	0.6	0.4
RT only	3	0.9	0.5	0.8
RT only	4	0.4	0.7	0.3
RT only	5	0.7	0.6	0.9
RT only	6	1.0	0.3	0.4
RT only	7	0.4	0.2	0.2
RT only	8	0.9	0.2	0.4
	Mean	**1.0**	**0.4**	**0.6**
	SD	**0.7**	**0.2**	**0.4**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.6	1.8	7.8
HIT+RT	2	0.9	1.1	0.5
HIT+RT	3	0.4	0.9	2.7
HIT+RT	4	0.8	0.7	2.0
HIT+RT	5	1.8	1.3	1.0
HIT+RT	6	0.7	0.9	0.7
HIT+RT	7	1.3	1.1	1.0
HIT+RT	8	0.6	0.6	0.8
	Mean	**1.0**	**1.0**	**2.1**
	SD	**0.5**	**0.4**	**2.4**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.2	1.4	1.0
MICT+RT	2	1.5	1.7	1.4
MICT+RT	3	1.4	0.7	0.8
MICT+RT	4	1.4	2.0	5.7
MICT+RT	5	0.3	0.2	0.8
MICT+RT	6	0.6	0.9	2.2
MICT+RT	7	0.6	0.9	0.7
	Mean	**1.0**	**1.1**	**1.8**
	SD	**0.5**	**0.6**	**1.8**

18S rRNA (mature)
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.0	0.5	1.1
RT only	2	1.4	1.1	0.7
RT only	3	0.8	0.7	0.9
RT only	4	1.1	1.7	0.6
RT only	5	1.2	0.7	1.2
RT only	6	1.4	0.8	0.6
RT only	7	0.0	1.1	0.6
RT only	8	1.1	0.7	0.8
	Mean	**1.0**	**0.9**	**0.8**
	SD	**0.4**	**0.4**	**0.2**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.5	1.0	4.0
HIT+RT	2	0.8	1.1	1.2
HIT+RT	3	0.5	0.6	1.1
HIT+RT	4	0.6	0.7	1.2
HIT+RT	5	1.3	1.1	0.9
HIT+RT	6	0.8	1.2	0.8
HIT+RT	7	1.6	1.1	1.2
HIT+RT	8	0.9	1.0	0.9
	Mean	**1.0**	**1.0**	**1.4**
	SD	**0.4**	**0.2**	**1.0**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	0.5	0.6	0.4
MICT+RT	2	0.6	0.8	0.4
MICT+RT	3	1.0	0.5	0.4
MICT+RT	4	3.6	0.6	0.7
MICT+RT	5	0.5	0.4	0.5
MICT+RT	6	0.4	0.5	0.8
MICT+RT	7	0.4	0.5	0.3
	Mean	**1.0**	**0.6**	**0.5**
	SD	**1.2**	**0.1**	**0.2**

28S rRNA (mature)
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.2	0.8	1.2
RT only	2	1.6	0.8	0.4
RT only	3	0.9	0.5	0.5
RT only	4	0.5	0.9	0.6
RT only	5	1.6	0.9	1.6
RT only	6	0.7	0.4	0.5
RT only	7	0.5	0.5	0.4
RT only	8	0.9	0.4	0.6
	Mean	**1.0**	**0.6**	**0.7**
	SD	**0.4**	**0.2**	**0.4**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.3	1.7	4.6
HIT+RT	2	0.9	1.3	0.7
HIT+RT	3	0.6	0.9	3.2
HIT+RT	4	0.9	1.0	1.4
HIT+RT	5	1.2	1.2	1.0
HIT+RT	6	0.8	1.0	0.6
HIT+RT	7	1.4	1.0	1.1
HIT+RT	8	0.8	1.0	0.9
	Mean	**1.0**	**1.1**	**1.7**
	SD	**0.3**	**0.3**	**1.4**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.1	1.2	0.9
MICT+RT	2	1.3	1.6	1.0
MICT+RT	3	1.2	0.8	0.7
MICT+RT	4	1.2	1.3	2.6
MICT+RT	5	0.7	0.8	0.9
MICT+RT	6	0.7	1.0	1.6
MICT+RT	7	0.7	0.9	0.7
	Mean	**1.0**	**1.1**	**1.2**
	SD	**0.3**	**0.3**	**0.7**

5.8S rRNA (span)
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.1	0.8	1.2
RT only	2	1.5	0.7	0.5
RT only	3	0.9	0.5	0.7
RT only	4	0.6	0.9	0.4
RT only	5	1.7	0.9	1.6
RT only	6	0.9	0.4	0.4
RT only	7	0.4	0.5	0.3
RT only	8	0.9	0.4	0.4
	Mean	**1.0**	**0.6**	**0.7**
	SD	**0.4**	**0.2**	**0.5**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.5	1.8	7.3
HIT+RT	2	1.1	1.4	1.4
HIT+RT	3	1.2	1.4	3.8
HIT+RT	4	1.0	0.7	1.6
HIT+RT	5	0.3	1.5	1.5
HIT+RT	6	1.0	N/A	1.2
HIT+RT	7	1.2	1.3	1.0
HIT+RT	8	0.8	0.9	0.9
	Mean	**1.0**	**1.3**	**2.3**
	SD	**0.4**	**0.4**	**2.2**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.0	1.3	1.0
MICT+RT	2	1.2	2.0	1.7
MICT+RT	3	1.4	0.6	0.8
MICT+RT	4	1.1	1.4	2.9
MICT+RT	5	1.1	0.4	1.0
MICT+RT	6	0.6	0.8	1.7
MICT+RT	7	0.6	0.9	0.6
	Mean	**1.0**	**1.1**	**1.4**
	SD	**0.3**	**0.6**	**0.8**

18S rRNA (span)
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.5	1.6	2.7
RT only	2	0.6	0.4	0.3
RT only	3	1.5	0.6	1.6
RT only	4	0.6	1.1	0.5
RT only	5	0.7	0.6	0.4
RT only	6	1.1	0.6	1.5
RT only	7	0.3	0.1	0.4
RT only	8	1.6	0.9	0.5
	Mean	**1.0**	**0.7**	**1.0**
	SD	**0.5**	**0.5**	**0.9**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.2	1.6	1.4
HIT+RT	2	0.4	0.4	0.1
HIT+RT	3	5.0	3.6	6.3
HIT+RT	4	0.2	0.1	0.3
HIT+RT	5	0.1	1.2	1.8
HIT+RT	6	0.5	0.3	0.3
HIT+RT	7	0.2	0.3	2.2
HIT+RT	8	0.3	0.6	0.9
	Mean	**1.0**	**1.0**	**1.7**
	SD	**1.6**	**1.2**	**2.0**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	0.6	0.3	0.5
MICT+RT	2	0.6	0.7	2.0
MICT+RT	3	2.3	1.8	1.5
MICT+RT	4	3.1	2.7	3.0
MICT+RT	5	0.1	0.1	0.2
MICT+RT	6	0.2	0.3	0.9
MICT+RT	7	0.1	0.1	0.2
	Mean	**1.0**	**0.9**	**1.2**
	SD	**1.2**	**1.0**	**1.0**

28S rRNA (span)
Data expressed as fold change from PRE (AU)

Group	Participant	PRE	POST	+3 h
RT only	1	1.2	0.8	1.2
RT only	2	1.6	0.8	0.4
RT only	3	0.9	0.5	0.5
RT only	4	0.5	0.9	0.6
RT only	5	1.6	0.9	1.6
RT only	6	0.7	0.4	0.5
RT only	7	0.5	0.5	0.4
RT only	8	0.9	0.4	0.6
	Mean	**1.0**	**0.6**	**0.7**
	SD	**0.4**	**0.2**	**0.4**

Group	Participant	PRE	POST	+3 h
HIT+RT	1	1.3	1.7	4.6
HIT+RT	2	0.9	1.3	0.7
HIT+RT	3	0.6	0.9	3.2
HIT+RT	4	0.9	1.0	1.4
HIT+RT	5	1.2	1.2	1.0
HIT+RT	6	0.8	1.0	0.6
HIT+RT	7	1.4	1.0	1.1
HIT+RT	8	0.8	1.0	0.9
	Mean	**1.0**	**1.1**	**1.7**
	SD	**0.3**	**0.3**	**1.4**

Group	Participant	PRE	POST	+3 h
MICT+RT	1	1.1	1.2	0.9
MICT+RT	2	1.3	1.6	1.0
MICT+RT	3	1.2	0.8	0.7
MICT+RT	4	1.2	1.3	2.6
MICT+RT	5	0.7	0.8	0.9
MICT+RT	6	0.7	1.0	1.6
MICT+RT	7	0.7	0.9	0.7
	Mean	**1.0**	**1.1**	**1.2**
	SD	**0.3**	**0.3**	**0.7**

Study 2: Immunohistochemical data

Type I muscle fibre CSA (μm^2)

Group	Participant	PRE	POST	Δ PRE (μm^2)
RT only	1	5718.5	6563.3	844.8
RT only	2	4747.3	7863.5	3116.2
RT only	3	4608.8	5400.6	791.8
RT only	4	5411.1	7632.4	2221.4
RT only	5	3315.0	3588.5	273.5
RT only	6	4016.1	2962.3	-1053.8
RT only	7	3952.6	4720.7	768.1
RT only	8	4537.7	Insufficient sample	-
	Mean	**4538.5**	**5533.1**	**994.6**
	SD	**848.1**	**1913.4**	**1343.9**

Group	Participant	PRE	POST	Δ PRE (μm^2)
HIT+RT	1	4535.6	3324.9	-1210.7
HIT+RT	2	5618.0	6854.9	1237.0
HIT+RT	3	5600.7	5274.9	-325.9
HIT+RT	4	8543.6	6462.8	-2080.8
HIT+RT	5	5372.5	6339.7	967.3
HIT+RT	6	8100.2	3943.6	-4156.6
HIT+RT	7	Insufficient sample		-
HIT+RT	8	9217.6	4082.4	-5135.2
	Mean	**6712.6**	**5183.3**	**-1529.3**
	SD	**1849.4**	**1413.0**	**2437.3**

Group	Participant	PRE	POST	Δ PRE (μm^2)
MICT+RT	1	5144.1	6012.6	868.5
MICT+RT	2	Insufficient sample	5770.8	-
MICT+RT	3	7244.9	4951.0	-2293.9
MICT+RT	4	2075.2	7442.6	5367.4
MICT+RT	5	4072.2	4535.3	463.1
MICT+RT	6	5855.2	3719.5	-2135.6
MICT+RT	7	8661.3	4163.9	-4497.4
	Mean	**5508.8**	**5228.0**	**-371.3**
	SD	**2326.3**	**1276.7**	**3435.1**

Type II muscle fibre CSA (μm^2)

Group	Participant	PRE	POST	Δ PRE (μm^2)
RT only	1	7349.3	7565.3	216.0
RT only	2	5037.7	9660.0	4622.3
RT only	3	6251.4	6405.9	154.5
RT only	4	6210.9	8548.5	2337.6
RT only	5	4235.8	4646.4	410.6
RT only	6	4394.2	3694.5	-699.7
RT only	7	3590.1	4667.9	1077.7
RT only	8	4598.7	Insufficient sample	-
	Mean	**5295.6**	**6455.5**	**1159.9**
	SD	**1346.6**	**2235.1**	**1793.6**

Group	Participant	PRE	POST	Δ PRE (μm^2)
HIT+RT	1	5167.2	3809.3	-1358.0
HIT+RT	2	4971.7	8171.9	3200.1
HIT+RT	3	5261.1	5454.9	193.8
HIT+RT	4	8673.8	9902.9	1229.1
HIT+RT	5	7294.0	7063.3	-230.8
HIT+RT	6	7891.4	5285.0	-2606.4
HIT+RT	7	Insufficient sample		-
HIT+RT	8	6032.0	6660.3	628.3
	Mean	**6470.2**	**6621.1**	**150.9**
	SD	**1480.5**	**2017.6**	**1858.1**

Group	Participant	PRE	POST	Δ PRE (μm^2)
MICT+RT	1	6036.2	6722.5	686.3
MICT+RT	2	Insufficient sample	6297.2	-
MICT+RT	3	6742.9	5641.3	-1101.5
MICT+RT	4	2627.7	5841.5	3213.8
MICT+RT	5	4249.0	5327.1	1078.0
MICT+RT	6	6106.5	5695.4	-411.1
MICT+RT	7	4542.9	4568.3	25.4
	Mean	**5050.9**	**5727.6**	**581.8**
	SD	**1531.1**	**688.0**	**1504.2**

Study 2: Post-training single-bout exercise trial data

Venous blood lactate (mmol·L^{-1})

		Time point during exercise																
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
1	HIT+RT	0.5	2.5	4.3	6.0	7.4	7.9	7.8	7.4	5.9	5.5	6.3	7.0	6.4	5.3	2.2	1.3	0.8	0.6
2	HIT+RT	1.2	3.4	7.4	8.2	9.6	8.5	9.9	9.2	8.3	6.6	2.6	2.7	2.6	2.1	1.2	1.3	0.6	0.8
3	HIT+RT	0.7	2.0	4.5	6.9	6.8	7.5	6.6	6.3	5.6	3.9	3.2	3.2	3.2	2.1	1.4	0.8	0.9	0.7
4	HIT+RT	0.5	2.8	7.1	7.8	8.4	8.3	8.8	8.5	6.8	6.3	3.2	2.9	2.9	2.3	1.9	1.2	0.7	0.6
5	HIT+RT	0.6	2.1	4.5	6.6	6.6	6.6	7.0	7.8	6.4	5.9	3.8	4.3	4.0	3.0	1.8	1.5	0.8	0.8
6	HIT+RT	0.8	3.3	6.4	6.8	6.9	7.5	7.6	7.6	6.4	4.6	2.4	2.5	2.5	2.4	1.3	1.2	0.8	0.7
7	HIT+RT	0.6	2.2	3.6	4.3	4.9	4.5	3.9	4.0	3.1	2.5	2.5	2.3	1.9	1.5	1.2	0.9	0.7	0.6
8	HIT+RT	1.0	2.3	5.2	7.7	7.7	7.1	7.0	6.8	5.3	4.2	3.9	3.9	3.0	2.1	1.5	1.6	0.9	0.7
Mean		**0.7**	**2.6**	**5.4**	**6.8**	**7.3**	**7.2**	**7.3**	**7.2**	**6.0**	**4.9**	**3.5**	**3.6**	**3.3**	**2.6**	**1.6**	**1.2**	**0.8**	**0.7**
SD		**0.3**	**0.5**	**1.4**	**1.2**	**1.4**	**1.3**	**1.7**	**1.6**	**1.5**	**1.4**	**1.3**	**1.5**	**1.4**	**1.2**	**0.4**	**0.3**	**0.1**	**0.1**

		Time point during exercise																
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
1	MICT+RT	1.2	1.6	2.9	2.6	2.6	2.4	2.0	1.8	1.7	1.4	2.3	2.1	1.8	1.7	0.8	0.7	0.7	0.4
2	MICT+RT	0.7	1.9	2.9	2.9	3.4	3.6	2.6	3.1	2.4	1.8	3.7	4.4	3.8	2.7	1.4	1.0	0.7	0.5
3	MICT+RT	0.8	2.0	2.2	2.1	1.8	1.8	1.6	1.4	1.1	0.8	1.1	1.0	0.8	0.8	0.5	0.5	0.6	0.6
4	MICT+RT	0.8	2.6	3.5	3.7	3.8	4.1	3.5	2.8	2.2	1.6	3.6	3.5	3.0	2.1	0.9	1.1	0.8	
5	MICT+RT	0.5	1.9	3.7	4.0	3.9	4.1	3.2	3.2	2.7	2.1	1.2	1.3	1.3	1.2	0.9	0.7	0.6	0.8
6	MICT+RT	0.3	1.0	1.6	2.2	2.3	2.2	1.7	1.3	0.9	0.8	3.3	3.8	3.6	2.4	1.2	0.6	0.5	0.4
7	MICT+RT	0.5	1.2	1.7	1.8	1.7	1.8	2.0	1.5	1.4	1.4	1.2	1.1	1.3	1.1	0.7	0.7	0.6	0.5
Mean		**0.7**	**1.7**	**2.6**	**2.7**	**2.8**	**2.8**	**2.4**	**2.2**	**1.8**	**1.4**	**2.4**	**2.5**	**2.2**	**1.7**	**0.9**	**0.7**	**0.6**	**0.5**
SD		**0.3**	**0.5**	**0.8**	**0.8**	**0.9**	**1.1**	**0.8**	**0.8**	**0.7**	**0.5**	**1.2**	**1.4**	**1.2**	**0.7**	**0.3**	**0.2**	**0.1**	**0.2**

		Time point during exercise								
Participant	**Trial**	**Rest**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
1	RT only	0.8	3.2	3.8	3.8	2.6	4.3	0.6	0.7	0.6
2	RT only	0.8	2.4	1.9	2.0	1.6	0.9	0.8	0.7	0.7
3	RT only	0.9	1.0	1.3	1.4	1.0	0.7	0.6	0.6	0.4
4	RT only	0.8	2.8	3.7	3.8	3.3	1.8	1.2	0.9	0.9
5	RT only	0.6	2.1	2.1	1.8	1.3	0.5	0.5	0.3	0.3
6	RT only	0.5	1.8	1.8	1.2	1.0	0.5	0.4	0.4	0.3
7	RT only	0.6	1.9	2.0	1.8	1.5	0.9	0.7	0.5	0.6
8	RT only	0.6	1.4	1.7	2.0	1.5	0.9	1.0	0.8	0.6
Mean		**0.7**	**2.1**	**2.3**	**2.2**	**1.7**	**1.3**	**0.7**	**0.6**	**0.5**
SD		**0.2**	**0.7**	**0.9**	**1.0**	**0.8**	**1.3**	**0.3**	**0.2**	**0.2**

Venous blood glucose (mmol·L^{-1})

		Time point during exercise																
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
1	HIT+RT	5.7	5.8	5.9	5.9	5.8	6.2	6.7	6.6	6.5	5.6	6.3	4.6	4.7	4.9	4.5	4.5	4.6	4.5
2	HIT+RT	3.7	4.0	4.8	5.2	6.2	6.9	8.4	7.9	7.2	6.7	3.7	4.0	4.1	4.1	4.6	4.5	4.7	4.6
3	HIT+RT	4.5	4.3	4.6	5.1	4.9	5.2	5.4	5.4	5.5	5.1	4.2	4.7	4.9	4.5	4.6	4.7	4.5	4.6
4	HIT+RT	6.0	6.0	5.8	5.8	6.8	6.8	7.6	7.5	6.8	6.2	4.9	5.0	5.1	4.8	4.8	5.0	4.8	5.1
5	HIT+RT	4.1	3.5	3.3	3.3	3.4	3.6	3.8	3.9	3.7	3.8	3.9	4.2	3.9	3.9	4.0	4.3	4.1	4.0
6	HIT+RT	5.0	4.8	5.3	5.6	5.9	7.5	7.3	7.2	6.9	6.3	4.6	4.7	4.7	4.6	4.3	4.7	4.4	4.7
7	HIT+RT	4.4	4.4	4.3	4.3	4.8	5.0	5.1	5.3	4.5	4.9	4.4	4.7	4.4	4.4	4.5	4.5	4.6	4.5
8	HIT+RT	4.2	4.0	4.6	5.1	5.8	5.9	6.1	6.1	6.1	4.6	3.8	4.0	4.4	4.1	4.4	5.1	4.5	4.8
Mean		**0.7**	**2.6**	**5.4**	**6.8**	**7.3**	**7.2**	**7.3**	**7.2**	**6.0**	**4.9**	**3.5**	**3.6**	**3.3**	**2.6**	**1.6**	**1.2**	**0.8**	**0.7**
SD		**0.3**	**0.5**	**1.4**	**1.2**	**1.4**	**1.3**	**1.7**	**1.6**	**1.5**	**1.4**	**1.3**	**1.5**	**1.4**	**1.2**	**0.4**	**0.3**	**0.1**	**0.1**

		Time point during exercise																
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**	**+2 min**	**+5 min**	**+10 min**	**+15 min**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
1	MICT+RT	5.3	5.2	5.4	4.2	4.2	4.1	4.2	4.5	4.6	4.6	5.0	4.7	4.7	4.9	4.8	4.5	4.5	4.6
2	MICT+RT	4.8	4.7	4.6	4.5	4.6	4.6	4.5	4.9	4.8	4.7	4.9	5.1	4.9	4.5	4.7	4.5	4.6	4.4
3	MICT+RT	4.5	4.6	4.5	4.5	4.3	4.3	4.4	4.5	4.5	4.4	4.7	4.4	4.6	4.5	4.5	4.2	4.3	4.0
4	MICT+RT	4.6	4.7	4.5	4.4	4.5	4.5	4.6	4.7	4.6	4.7	4.5	4.6	4.5	4.5	4.6	4.4	4.4	
5	MICT+RT	4.7	4.6	4.5	4.4	4.9	5.1	5.4	5.6	5.3	5.2	4.6	4.7	4.9	4.7	5.0	4.6	4.7	5.1
6	MICT+RT	3.6	4.0	3.8	3.9	3.8	3.9	4.0	4.3	4.0	3.7	4.4	4.8	4.8	4.6	4.7	4.3	4.2	4.0
7	MICT+RT	4.2	4.0	3.6	3.7	4.0	3.8	4.1	4.3	4.1	4.3	4.3	4.2	4.4	4.3	4.7	4.5	4.5	4.6
Mean		**0.7**	**1.7**	**2.6**	**2.7**	**2.8**	**2.8**	**2.4**	**2.2**	**1.8**	**1.4**	**2.4**	**2.5**	**2.2**	**1.7**	**0.9**	**0.7**	**0.6**	**0.5**
SD		**0.3**	**0.5**	**0.8**	**0.8**	**0.9**	**1.1**	**0.8**	**0.8**	**0.7**	**0.5**	**1.2**	**1.4**	**1.2**	**0.7**	**0.3**	**0.2**	**0.1**	**0.2**

		Time point during exercise								
Participant	**Trial**	**Rest**	**End leg press**	**+2 min**	**+5 min**	**+10 min**	**+30 min**	**+60 min**	**+90 min**	**+180 min**
1	RT only	5.0	4.6	4.6	4.7	4.8	4.9	3.2	4.6	4.5
2	RT only	4.4	4.5	4.4	4.5	4.6	4.5	4.6	4.7	4.6
3	RT only	4.9	5.2	5.3	5.5	5.3	5.1	4.6	4.8	4.5
4	RT only	5.3	5.2	5.4	5.3	5.6	5.0	5.0	4.6	4.4
5	RT only	4.6	4.4	4.5	4.5	4.3	4.6	4.2	3.9	4.3
6	RT only	4.5	4.7	4.6	4.5	4.5	4.4	4.2	4.4	4.5
7	RT only	4.4	4.6	4.7	4.4	4.5	4.7	4.3	4.3	4.9
8	RT only	4.4	4.4	4.4	4.6	4.5	4.5	4.3	4.5	4.8
Mean		**0.7**	**2.1**	**2.3**	**2.2**	**1.7**	**1.3**	**0.7**	**0.6**	**0.5**
SD		**0.2**	**0.7**	**0.9**	**1.0**	**0.8**	**1.3**	**0.3**	**0.2**	**0.2**

Heart rate (beats·min^{-1})

		Time point during cycling					
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
1	HIT+RT	72	154	160	168	175	176
2	HIT+RT	48	171	178	182	187	187
3	HIT+RT	67	150	162	168	171	175
4	HIT+RT	72	148	158	160	162	168
5	HIT+RT	65	145	154	160	162	166
6	HIT+RT	58	162	169	169	176	179
7	HIT+RT	75	147	150	152	156	158
8	HIT+RT	45	157	167	171	167	172
	Mean	**63**	**154**	**162**	**166**	**170**	**173**
	SD	**11**	**9**	**9**	**9**	**10**	**9**

		Time point during cycling					
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
1	MICT+RT	72	139	145	144	144	148
2	MICT+RT	65	148	158	160	160	161
3	MICT+RT	66	136	140	144	148	152
4	MICT+RT	72	164	165	169	169	171
5	MICT+RT	56	140	146	150	154	155
6	MICT+RT	65	140	158	162	169	172
7	MICT+RT	68	111	115	118	119	121
	Mean	**66**	**140**	**147**	**150**	**152**	**154**
	SD	**5**	**16**	**17**	**17**	**17**	**17**

Rating of perceived exertion (RPE) (AU)

		Time point during cycling					
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
1	HIT+RT	6	15	17	18	19	19
2	HIT+RT	6	15	17	19	20	20
3	HIT+RT	6	11	12	13	14	15
4	HIT+RT	6	13	17	20	20	20
5	HIT+RT	6	11	13	16	18	19
6	HIT+RT	6	11	13	15	15	15
7	HIT+RT	6	9	12	15	17	19
8	HIT+RT	6	16	18	19	20	20
	Mean	**6**	**13**	**15**	**17**	**18**	**18**
	SD	**0**	**3**	**3**	**2**	**2**	**2**

		Time point during cycling					
Participant	**Trial**	**Rest**	**10 min**	**16 min**	**22 min**	**28 min**	**34 min**
1	MICT+RT	6	11	12	12	12	12
2	MICT+RT	6	14	15	15	16	16
3	MICT+RT	6	11	12	12	12	12
4	MICT+RT	6	11	13	15	16	16
5	MICT+RT	6	8	9	10	11	11
6	MICT+RT	6	12	12	12	13	13
7	MICT+RT	6	13	14	15	15	15
	Mean	**6**	**11**	**12**	**13**	**14**	**14**
	SD	**0**	**2**	**2**	**2**	**2**	**2**

Appendix M: Within-trial comparison data for Study 1 (Chapter 3)[9] [10]

[9] Within-trial comparisons for Study 1 are only reported for the RE trial as this was the only trial in which a true resting muscle biopsy obtained.
[10] Magnitude-based inference data were calculated using 0.40 as the smallest worthwhile effect

Measure	Trial	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
Muscle glycogen	RE	PRE - +1 h	10	13	0.41	0.48	small	possibly	0.260
		PRE - +3 h	1.6	11	0.07	0.45	trivial	unlikely	0.791
		+1 h - + 3 h	-7.9	7.5	-0.34	0.33	small	most unlikely	0.146
p-mTOR	RE	PRE - +1 h	37	38	0.24	0.21	small	unlikely	0.163
		PRE - +3 h	-14	59	-0.11	0.48	trivial	unlikely	0.658
		+1 h - + 3 h	-37	35	-0.35	0.40	small	possibly	0.126
p-p70S6K1	RE	PRE - +1 h	171	95	1.39	0.48	large	most likely	0.002
		PRE - +3 h	65	56	0.70	0.47	moderate	likely	0.025
		+1 h - + 3 h	-39	27	-0.70	0.61	moderate	likely	0.056
p-4E-BP1	RE	PRE - +1 h	9	20	0.07	0.14	trivial	most unlikely	0.379
		PRE - +3 h	-18	18	-0.16	0.18	trivial	very unlikely	0.471
		+1 h - + 3 h	-25	20	-0.23	0.21	small	unlikely	0.364
p-eEF2	RE	PRE - +1 h	-10	21	-0.43	0.94	small	possibly	0.472
		PRE - +3 h	-20	39	-0.93	1.92	moderate	possibly	0.403
		+1 h - + 3 h	-11	24	-0.50	1.12	moderate	possibly	0.400
p-rps6	RE	PRE - +1 h	215	155	1.21	0.50	moderate	very likely	0.029
		PRE - +3 h	203	226	1.17	0.73	moderate	very likely	0.018
		+1 h - + 3 h	-4	111	-0.04	1.04	trivial	possibly	0.941

Measure	Trial	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-GSK-3β	RE	PRE - +1 h	130	87	0.70	0.31	moderate	likely	0.002
		PRE - +3 h	59	59	0.39	0.30	small	possibly	0.118
		+1 h - + 3 h	-31	23	-0.31	0.27	small	possibly	0.144
p-ACC	RE	PRE - +1 h	-60	24	-1.15	0.70	large	very likely	0.017
		PRE - +3 h	-82	28	-2.15	1.53	very large	very likely	0.040
		+1 h - + 3 h	-55	35	-1.00	0.90	moderate	likely	0.093
p-AMPK	RE	PRE - +1 h	137	162	1.12	0.83	moderate	likely	0.033
		PRE - +3 h	201	143	1.43	0.60	large	very likely	0.002
		+1 h - + 3 h	27	83	0.31	0.80	small	possibly	0.479

mRNA target	Trial	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
MuRF-1	RE	PRE - +3 h	-58	96	-0.29	0.53	small	possibly	0.292
Atrogin-1	RE	PRE - +3 h	-25	30	-0.11	0.15	trivial	very unlikely	0.419
PGC-1α	RE	PRE - +3 h	49	57	0.39	0.37	small	possibly	0.284
TSC2	RE	PRE - +3 h	37	199	0.13	0.47	trivial	unlikely	0.669
Rheb	RE	PRE - +3 h	73	118	0.37	0.44	small	possibly	0.228
Myostatin	RE	PRE - +3 h	63	69	0.26	0.22	small	unlikely	0.120
Fox-O1	RE	PRE - +3 h	-9	57	-0.07	0.47	trivial	very unlikely	0.772

microRNA target	Trial	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
miR-1	RE	PRE - +1 h	53	35	0.44	0.24	small	possibly	0.241
		PRE - +3 h	18	17	0.17	0.15	trivial	very unlikely	0.317
miR-133a	RE	PRE - +1 h	-25	13	-0.35	0.21	small	possibly	0.144
		PRE - +3 h	-27	14	-0.38	0.23	small	possibly	0.026
miR-378	RE	PRE - +1 h	-15	15	-0.27	0.30	small	unlikely	0.259
		PRE - +3 h	-5	10	-0.09	0.19	trivial	very unlikely	0.526
miR-486	RE	PRE - +1 h	8	16	0.17	0.33	trivial	unlikely	0.677
		PRE - +3 h	-2	13	-0.05	0.31	trivial	very unlikely	0.814

Appendix N: Between-trial comparison data for Study 1 (Chapter 3)[11]

[11] Magnitude-based inference data were calculated using 0.40 as the smallest worthwhile effect

Measure	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
Muscle glycogen	PRE/POST	HIT+RE vs. RE	-50	23	-2.91	1.86	very large	very likely	0.022
		MICT+RE vs. RE	-40	11	-2.10	0.76	very large	most likely	0.004
		HIT+RE vs. MICT+RE	21	58	0.81	1.92	moderate	unlikely	0.410
	+1 h	HIT+RE vs. RE	-62	15	-4.01	1.56	extremely large	most likely	0.002
		MICT+RE vs. RE	-43	19	-2.36	1.38	very large	very likely	0.014
		HIT+RE vs. MICT+RE	-33	22	-1.65	1.32	large	likely	0.034
	+3 h	HIT+RE vs. RE	-45	11	-2.46	0.80	very large	most likely	0.001
		MICT+RE vs. RE	-31	18	-1.55	1.07	large	very likely	0.033
		HIT+RE vs. MICT+RE	-20	33	-0.91	1.68	moderate	possibly	0.341
p-mTOR	PRE/POST	HIT+RE vs. RE	105	34	0.84	0.19	moderate	most likely	0.011
		MICT+RE vs. RE	15	47	0.16	0.46	trivial	unlikely	0.632
		HIT+RE vs. MICT+RE	-44	21	-0.67	0.44	moderate	likely	0.014
	+1 h	HIT+RE vs. RE	41	49	0.35	0.34	small	possibly	0.227
		MICT+RE vs. RE	38	25	-0.48	0.40	small	possibly	0.054
		HIT+RE vs. MICT+RE	128	67	0.83	0.29	moderate	very likely	0.003
	+3 h	HIT+RE vs. RE	91	84	0.75	0.49	moderate	likely	0.148
		MICT+RE vs. RE	19	101	-0.25	1.22	small	possibly	0.714
		HIT+RE vs. MICT+RE	138	285	1.01	1.18	moderate	likely	0.150

Measure	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-p70S6K1	PRE/POST	HIT+RE vs. RE	77	55	0.80	0.43	moderate	likely	0.154
		MICT+RE vs. RE	60	67	0.66	0.57	moderate	likely	0.178
		HIT+RE vs. MICT+RE	9	66	-0.14	0.95	small	possibly	0.772
	+1 h	HIT+RE vs. RE	0	31	0.00	0.43	trivial	unlikely	0.996
		MICT+RE vs. RE	2	34	0.03	0.45	trivial	unlikely	0.918
		HIT+RE vs. MICT+RE	2	31	-0.03	0.43	trivial	unlikely	0.910
	+3 h	HIT+RE vs. RE	19	40	0.24	0.46	small	possibly	0.457
		MICT+RE vs. RE	8	37	-0.11	0.55	trivial	unlikely	0.783
		HIT+RE vs. MICT+RE	29	79	0.36	0.81	small	possibly	0.433
p-4E-BP1	PRE/POST	HIT+RE vs. RE	18	25	-0.16	0.24	trivial	possibly	0.449
		MICT+RE vs. RE	2	34	0.01	0.27	trivial	unlikely	0.952
		HIT+RE vs. MICT+RE	25	33	0.18	0.21	trivial	possibly	0.119
	+1 h	HIT+RE vs. RE	10	28	-0.08	0.25	trivial	unlikely	0.663
		MICT+RE vs. RE	2	32	0.02	0.25	trivial	unlikely	0.904
		HIT+RE vs. MICT+RE	12	21	-0.10	0.19	trivial	unlikely	0.320
	+3 h	HIT+RE vs. RE	19	57	0.14	0.37	trivial	possibly	0.453
		MICT+RE vs. RE	41	66	0.27	0.36	small	possibly	0.169

Measure	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-eEF2	PRE/POST	HIT+RE vs. RE	34	33	-1.72	1.98	large	likely	0.114
		MICT+RE vs. RE	37	23	-1.87	1.47	large	likely	0.035
		HIT+RE vs. MICT+RE	4	79	-0.15	3.06	trivial	possibly	0.922
	+1 h	HIT+RE vs. RE	16	10	-0.71	0.49	moderate	likely	0.050
		MICT+RE vs. RE	21	24	-0.99	1.22	moderate	likely	0.179
		HIT+RE vs. MICT+RE	7	29	0.28	1.11	small	possibly	0.624
	+3 h	HIT+RE vs. RE	11	23	-0.47	1.03	small	possibly	0.671
		MICT+RE vs. RE	13	17	-0.57	0.80	small	possibly	0.562
		HIT+RE vs. MICT+RE	2	29	0.09	1.14	trivial	possibly	0.883
p-rps6	PRE/POST	HIT+RE vs. RE	153	116	0.98	0.47	moderate	very likely	0.023
		MICT+RE vs. RE	56	98	0.47	0.63	moderate	possibly	0.210
		HIT+RE vs. MICT+RE	38	36	-0.51	0.59	moderate	possibly	0.128
	+1 h	HIT+RE vs. RE	9	100	0.09	0.87	trivial	possibly	0.848
		MICT+RE vs. RE	21	73	-0.24	0.87	small	possibly	0.635
		HIT+RE vs. MICT+RE	37	68	0.33	0.50	small	possibly	0.216
	+3 h	HIT+RE vs. RE	22	113	0.21	0.87	small	possibly	0.680
		MICT+RE vs. RE	26	62	-0.32	0.81	small	possibly	0.491
		HIT+RE vs. MICT+RE	65	188	0.53	1.03	small	possibly	0.365

Measure	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-GSK-3β	PRE/POST	HIT+RE vs. RE	73	103	0.46	0.47	small	possibly	0.151
		MICT+RE vs. RE	63	100	0.41	0.49	small	possibly	0.121
		HIT+RE vs. MICT+RE	6	79	-0.05	0.64	trivial	unlikely	0.894
	+1 h	HIT+RE vs. RE	1	37	0.01	0.30	trivial	very unlikely	0.965
		MICT+RE vs. RE	23	30	-0.22	0.10	small	unlikely	0.410
		HIT+RE vs. MICT+RE	31	56	0.23	0.58	small	unlikely	0.329
	+3 h	HIT+RE vs. RE	6	59	0.05	0.45	trivial	unlikely	0.845
		MICT+RE vs. RE	18	49	-0.17	0.48	trivial	unlikely	0.493
		HIT+RE vs. MICT+RE	30	81	0.22	0.50	small	possibly	0.434
p-ACC	PRE/POST	HIT+RE vs. RE	530	145	2.29	0.28	very large	most likely	< 0.001
		MICT+RE vs. RE	451	274	2.13	0.60	very large	most likely	0.002
		HIT+RE vs. MICT+RE	12	34	-0.17	0.47	trivial	unlikely	0.482
	+1 h	HIT+RE vs. RE	133	96	1.06	0.50	moderate	very likely	0.038
		MICT+RE vs. RE	11	40	-0.14	0.54	trivial	unlikely	0.821
		HIT+RE vs. MICT+RE	161	218	1.19	0.95	moderate	likely	0.046
	+3 h	HIT+RE vs. RE	458	215	2.14	0.47	very large	most likely	0.043
		MICT+RE vs. RE	114	138	0.95	0.76	moderate	likely	0.331
		HIT+RE vs. MICT+RE	161	121	1.20	0.56	large	very likely	0.005

Measure	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-AMPK	PRE/POST	HIT+RE vs. RE	107	163	0.94	0.94	moderate	likely	0.144
		MICT+RE vs. RE	118	160	1.01	0.88	moderate	likely	0.074
		HIT+RE vs. MICT+RE	6	180	0.07	1.69	trivial	possibly	0.933
	+1 h	HIT+RE vs. RE	48	86	0.51	0.72	small	possibly	0.261
		MICT+RE vs. RE	59	61	0.61	0.48	moderate	likely	0.085
		HIT+RE vs. MICT+RE	7	51	-0.10	0.68	trivial	unlikely	0.777
	+3 h	HIT+RE vs. RE	20	46	-0.29	0.70	small	possibly	0.500
		MICT+RE vs. RE	20	26	-0.30	0.42	small	possibly	0.622
		HIT+RE vs. MICT+RE	1	80	0.01	0.95	trivial	unlikely	0.983

mRNA target	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
MuRF-1 mRNA	PRE/POST	HIT+RE - RE	-83	28	-2.61	1.88	very large	very likely	0.103
		MICT+RE - RE	-81	81	-2.44	3.17	very large	likely	0.174
		HIT+RE - MICT+RE	12	861	0.17	4.02	trivial	possibly	0.934
	+3 h	HIT+RE - RE	585	684	0.52	0.64	small	likely	0.170
		MICT+RE - RE	535	463	0.33	0.20	small	likely	0.016
		HIT+RE - MICT+RE	-82	58	-0.41	0.45	small	possibly	0.130
Atrogin-1 mRNA	PRE/POST	HIT+RE - RE	-58	67	-0.40	0.57	small	possibly	0.272
		MICT+RE - RE	-35	121	-0.20	0.64	small	possibly	0.638
		HIT+RE - MICT+RE	53	426	0.20	0.81	small	possibly	0.630
	+3 h	HIT+RE - RE	55	138	-0.37	0.86	small	possibly	0.407
		MICT+RE - RE	7	118	-0.03	0.49	trivial	unlikely	0.889
		HIT+RE - MICT+RE	52	72	-0.34	0.55	small	possibly	0.283
PGC-1α mRNA	PRE/POST	HIT+RE - RE	42	42	-0.27	0.40	small	possibly	0.446
		MICT+RE - RE	35	35	-0.21	0.63	small	possibly	0.628
		HIT+RE - MICT+RE	12	12	0.05	0.74	trivial	unlikely	0.884
	+3 h	HIT+RE - RE	788	878	0.54	0.54	small	likely	0.098
		MICT+RE - RE	604	403	0.59	0.42	small	likely	0.033
		HIT+RE - MICT+RE	64	64	-0.25	0.29	small	unlikely	0.152

mRNA target	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
Rheb mRNA	PRE/POST	HIT+RE - RE	-41	72	-0.44	0.86	small	possibly	0.345
		MICT+RE - RE	-21	128	-0.20	1.06	small	possibly	0.714
		HIT+RE - MICT+RE	61	232	0.40	0.98	small	possibly	0.420
	+3 h	HIT+RE - RE	-32	44	-0.39	0.62	small	possibly	0.339
		MICT+RE - RE	16	55	0.15	0.46	trivial	unlikely	0.548
		HIT+RE - MICT+RE	-46	41	-0.62	0.70	moderate	possibly	0.138
TSC2 mRNA	PRE/POST	HIT+RE - RE	-32	313	-0.12	0.70	trivial	unlikely	0.657
		MICT+RE - RE	-43	781	-0.17	1.03	trivial	possibly	0.454
		HIT+RE - MICT+RE			0.40	5.24	small	possibly	0.577
	+3 h	HIT+RE - RE	56	339	0.21	0.72	small	unlikely	0.913
		MICT+RE - RE	99	185	0.33	0.40	small	possibly	0.257
		HIT+RE - MICT+RE	-43	105	-0.27	0.65	small	possibly	0.431
Myostatin mRNA	PRE/POST	HIT+RE - RE	82	75	0.48	0.32	small	possibly	0.075
		MICT+RE - RE	-15	16	-0.13	0.15	trivial	most unlikely	0.747
		HIT+RE - MICT+RE	-53	18	-0.60	0.30	moderate	likely	0.044
	+3 h	HIT+RE - RE	15	59	0.08	0.29	trivial	very unlikely	0.623
		MICT+RE - RE	-1	30	0.00	0.17	trivial	most unlikely	0.963
		HIT+RE - MICT+RE	16	92	0.09	0.43	trivial	unlikely	0.713

mRNA target	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
Fox-O1 mRNA	PRE/POST	HIT+RE - RE	-54	73	-0.43	0.69	small	possibly	0.302
		MICT+RE - RE	-53	34	-0.41	0.36	small	possibly	0.139
		HIT+RE - MICT+RE	3	276	0.02	0.95	trivial	unlikely	0.969
	+3 h	HIT+RE - RE	30	76	0.18	0.39	trivial	unlikely	0.558
		MICT+RE - RE	-20	65	-0.16	0.52	trivial	unlikely	0.665
		HIT+RE - MICT+RE	62	58	0.34	0.25	small	possibly	0.036

microRNA target	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
miR-1	PRE/POST	HIT+RE - RE	26	42	0.22	0.31	small	unlikely	0.633
		MICT+RE - RE	28	66	0.23	0.47	small	possibly	0.584
		HIT+RE - MICT+RE	1	60	0.01	0.53	trivial	unlikely	0.967
	+1 h	HIT+RE - RE	-38	40	-0.46	0.58	small	possibly	0.167
		MICT+RE - RE	-19	40	-0.19	0.44	trivial	unlikely	0.399
		HIT+RE - MICT+RE	-24	74	-0.26	0.82	small	possibly	0.526
	+3 h	HIT+RE - RE	11	40	0.10	0.33	trivial	unlikely	0.738
		MICT+RE - RE	32	46	0.26	0.32	small	unlikely	0.326
		HIT+RE - MICT+RE	-16	38	-0.16	0.41	trivial	unlikely	0.487
miR-133a	PRE/POST	HIT+RE - RE	-40	13	-0.63	0.27	small	likely	0.014
		MICT+RE - RE	-15	11	-0.19	0.15	small	very unlikely	0.473
		HIT+RE - MICT+RE	43	45	0.44	0.37	small	possibly	0.071
	+1 h	HIT+RE - RE	-35	28	-0.53	0.50	small	likely	0.068
		MICT+RE - RE	13	17	0.15	0.18	trivial	very unlikely	0.271
		HIT+RE - MICT+RE	-43	30	-0.68	0.61	moderate	likely	0.053
	+3 h	HIT+RE - RE	-15	21	-0.20	0.30	small	unlikely	0.290
		MICT+RE - RE	-10	17	-0.12	0.22	trivial	very unlikely	0.561
		HIT+RE - MICT+RE	-6	29	-0.08	0.37	trivial	unlikely	0.703

microRNA target	Timepoint	Group comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
miR-378	PRE/POST	HIT+RE - RE	-15	15	-0.27	0.30	small	unlikely	0.259
		MICT+RE - RE	-5	10	-0.09	0.19	trivial	very unlikely	0.526
		HIT+RE - MICT+RE	11	10	0.18	0.15	trivial	very unlikely	0.102
	+1 h	HIT+RE - RE	-37	26	-0.79	0.70	moderate	likely	0.052
		MICT+RE - RE	9	21	0.14	0.33	trivial	unlikely	0.401
		HIT+RE - MICT+RE	-42	28	-0.94	0.81	moderate	likely	0.046
	+3 h	HIT+RE - RE	-13	10	-0.23	0.19	small	unlikely	0.167
		MICT+RE - RE	-5	24	-0.08	0.43	trivial	unlikely	0.712
		HIT+RE - MICT+RE	-8	27	-0.15	0.49	trivial	unlikely	0.575
miR-486	PRE/POST	HIT+RE - RE	-11	16	-0.26	0.41	small	possibly	0.297
		MICT+RE - RE	-1	15	-0.03	0.34	trivial	very unlikely	0.876
		HIT+RE - MICT+RE	11	11	0.23	0.22	small	unlikely	0.102
	+1 h	HIT+RE - RE	-36	17	-1.02	0.59	moderate	very likely	0.040
		MICT+RE - RE	4	29	0.08	0.62	trivial	unlikely	0.798
		HIT+RE - MICT+RE	-38	15	-1.10	0.56	moderate	very likely	0.015
	+3 h	HIT+RE - RE	-9	18	-0.21	0.44	small	unlikely	0.444
		MICT+RE - RE	-1	30	-0.02	0.67	trivial	unlikely	0.956
		HIT+RE - MICT+RE	-8	43	-0.19	1.02	trivial	possibly	0.735

Appendix O: Within-group comparison data for Study 2 (Chapters 4 and 5)[12]

[12] Magnitude-based inference data were calculated using 0.20 as the smallest worthwhile effect for all performance and body composition measures, whereas 0.40 was used for all molecular data as per comparisons for Study 1 (Chapter 3)

Measure	Group	Mean PRE-POST change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
		% change	±90% CL	ES (*d*)	±90% CL			
Maximal strength								
1RM leg press	RT	38.5	8.5	1.26	0.24	large	most likely	< 0.001
	HIT+RT	28.7	5.3	1.17	0.19	moderate	most likely	< 0.001
	MICT+RT	27.5	4.6	0.81	0.12	moderate	most likely	0.001
1RM bench press	RT	20.5	6.2	0.50	0.14	small	most likely	< 0.001
	HIT+RT	15.9	2.6	0.62	0.09	moderate	most likely	< 0.001
	MICT+RT	14.8	2.3	0.39	0.06	small	most likely	< 0.001
Counter-movement jump (CMJ) variables								
Peak CMJ force	RT	7.4	3.4	0.46	0.20	small	very likely	0.008
	HIT+RT	0.1	3.6	0.00	0.23	trivial	unlikely	0.979
	MICT+RT	-0.8	4.9	-0.04	0.26	trivial	unlikely	0.790
Peak CMJ power	RT	12.6	10.5	1.09	0.85	moderate	very likely	0.035
	HIT+RT	3.2	5.6	0.20	0.34	small	possibly	0.266
	MICT+RT	5.0	6.1	0.19	0.23	trivial	possibly	0.241
Peak CMJ velocity	RT	9.6	8.2	0.29	0.24	small	likely	0.099
	HIT+RT	2.6	4.8	0.17	0.31	trivial	possibly	0.306
	MICT+RT	6.0	4.0	0.40	0.26	small	likely	0.015
Peak CMJ displacement	RT	9.5	10.0	0.22	0.22	small	possibly	0.108
	HIT+RT	7.8	9.1	0.50	0.56	small	likely	0.134
	MICT+RT	7.0	8.5	0.34	0.40	small	possibly	0.129

Measure	Group	Mean PRE-POST change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
		% change	±90% CL	ES (*d*)	±90% CL			
Body composition								
Lean mass (lower)	RT	4.1	2.0	0.33	0.16	small	likely	0.023
	HIT+RT	1.8	1.6	0.13	0.12	trivial	unlikely	0.069
	MICT+RT	3.6	2.4	0.45	0.30	small	likely	0.052
Lean mass (upper)	RT	0.4	1.9	0.02	0.12	trivial	very unlikely	0.719
	HIT+RT	1.4	2.0	0.13	0.17	trivial	unlikely	0.198
	MICT+RT	1.8	2.9	0.17	0.28	small	possibly	0.325
Lean mass (total)	RT	1.6	1.4	0.12	0.10	trivial	unlikely	0.102
	HIT+RT	1.6	1.1	0.14	0.09	trivial	unlikely	0.038
	MICT+RT	2.4	2.4	0.27	0.26	small	possibly	0.151
Body fat %	RT	-0.6	1.0	-0.08	0.17	trivial	unlikely	0.372
	HIT+RT	-0.2	0.9	-0.03	0.15	trivial	very unlikely	0.659
	MICT+RT	-0.9	1.0	-0.23	0.25	small	possibly	0.115

Measure	Group	Mean PRE-POST change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
		% change	±90% CL	ES (*d*)	±90% CL			
Aerobic capacity								
Absolute $\dot{V}O_{2peak}$	RT	-0.6	6.4	-0.02	0.21	trivial	unlikely	0.876
	HIT+RT	5.3	2.7	0.25	0.12	small	likely	0.162
	MICT+RT	6.1	5.0	0.27	0.22	small	possibly	0.103
Relative $\dot{V}O_{2peak}$	RT	-2.2	6.7	-0.06	0.17	trivial	unlikely	0.593
	HIT+RT	4.0	4.6	0.11	0.13	trivial	unlikely	0.320
	MICT+RT	5.0	5.4	0.18	0.18	trivial	possibly	0.131
Lactate threshold	RT	7.4	9.4	0.13	0.16	trivial	unlikely	0.161
	HIT+RT	8.3	6.5	0.20	0.15	small	possibly	0.054
	MICT+RT	12.6	8.0	0.30	0.18	small	likely	0.107
Peak aerobic power	RT	-2.2	6.5	-0.06	0.17	trivial	very unlikely	0.515
	HIT+RT	8.8	4.1	0.31	0.14	small	likely	0.010
	MICT+RT	4.9	4.8	0.19	0.18	trivial	possibly	0.096

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-mTOR	RT	PRE-POST	1	65	0.01	0.39	trivial	very unlikely	0.967
		PRE-+1 h	108	125	0.42	0.33	small	possibly	0.036
		PRE-+3 h	143	130	0.49	0.28	small	possibly	0.022
		POST-+1 h	105	137	0.46	0.40	small	possibly	0.048
		POST-+3 h	140	245	0.56	0.57	small	possibly	0.083
	HIT+RT	PRE-POST	9	31	0.10	0.33	trivial	unlikely	0.557
		PRE-+1 h	42	51	0.38	0.38	small	possibly	0.077
		PRE-+3 h	85	51	0.60	0.27	moderate	likely	0.031
		POST-+1 h	30	71	0.32	0.62	small	possibly	0.320
		POST-+3 h	70	45	0.64	0.31	moderate	likely	0.030
	MICT+RT	PRE-POST	-25	39	-0.18	0.33	trivial	unlikely	0.441
		PRE-+1 h	33	157	0.17	0.59	trivial	unlikely	0.553
		PRE-+3 h	16	67	0.07	0.28	trivial	very unlikely	0.707
		POST-+1 h	77	184	0.37	0.59	small	possibly	0.218
		POST-+3 h	53	46	0.28	0.19	small	unlikely	0.032
Total mTOR	RT	PRE-POST	5	9	0.07	0.12	trivial	most unlikely	0.740
		PRE-+1 h	20	28	0.23	0.29	small	unlikely	0.212
		PRE-+3 h	31	52	0.27	0.39	small	possibly	0.245
		POST-+1 h	14	24	0.18	0.28	trivial	unlikely	0.285
		POST-+3 h	25	42	0.30	0.44	small	possibly	0.212

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	HIT+RT	PRE-POST	6	15	0.09	0.20	trivial	very unlikely	0.455
		PRE-+1 h	-7	20	-0.08	0.27	trivial	very unlikely	0.533
		PRE-+3 h	-9	32	-0.10	0.36	trivial	unlikely	0.600
		POST-+1 h	-12	16	-0.18	0.26	trivial	unlikely	0.194
		POST-+3 h	-14	24	-0.21	0.39	small	unlikely	0.289
	MICT+RT	PRE-POST	29	37	0.43	0.48	small	possibly	0.104
		PRE-+1 h	17	32	0.23	0.42	small	unlikely	0.375
		PRE-+3 h	11	19	0.18	0.28	trivial	unlikely	0.291
		POST-+1 h	-9	16	-0.17	0.30	trivial	unlikely	0.551
		POST-+3 h	-14	30	-0.25	0.58	small	possibly	0.428
p-p70S6K1	RT	PRE-POST	46	84	0.33	0.48	small	possibly	0.248
		PRE-+1 h	160	58	0.74	0.17	moderate	most likely	< 0.001
		PRE-+3 h	210	191	0.84	0.43	moderate	very likely	0.013
		POST-+1 h	78	77	0.51	0.37	small	possibly	0.026
		POST-+3 h	112	209	0.66	0.76	moderate	possibly	0.115
	HIT+RT	PRE-POST	94	47	0.66	0.24	moderate	very likely	0.024
		PRE-+1 h	113	101	0.71	0.43	moderate	likely	0.065
		PRE-+3 h	117	92	0.62	0.33	moderate	likely	0.029
		POST-+1 h	10	53	0.09	0.47	trivial	unlikely	0.703
		POST-+3 h	12	45	0.11	0.39	trivial	unlikely	0.574

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	27	50	0.38	0.60	small	possibly	0.250
		PRE-+1 h	66	26	0.75	0.23	moderate	very likely	0.058
		PRE-+3 h	35	31	0.51	0.39	small	possibly	0.097
		POST-+1 h	31	63	0.42	0.74	small	possibly	0.321
		POST-+3 h	6	38	0.09	0.56	trivial	unlikely	0.734
Total-p70S6K1	RT	PRE-POST	3	12	0.06	0.23	trivial	very unlikely	0.602
		PRE-+1 h	-10	12	-0.19	0.24	trivial	unlikely	0.211
		PRE-+3 h	-17	22	-0.30	0.41	small	possibly	0.261
		POST-+1 h	-13	16	-0.27	0.36	small	possibly	0.181
		POST-+3 h	-20	20	-0.44	0.49	small	possibly	0.168
	HIT+RT	PRE-POST	10	13	0.27	0.35	small	possibly	0.277
		PRE-+1 h	-7	15	-0.18	0.40	trivial	unlikely	0.505
		PRE-+3 h	-9	9	-0.22	0.24	small	unlikely	0.091
		POST-+1 h	-15	10	-0.47	0.33	small	possibly	0.020
		POST-+3 h	-17	7	-0.55	0.24	small	likely	0.026
	MICT+RT	PRE-POST	-19	43	-0.22	0.53	small	possibly	0.400
		PRE-+1 h	-9	28	-0.09	0.29	trivial	very unlikely	0.560
		PRE-+3 h	6	17	0.06	0.18	trivial	very unlikely	0.513
		POST-+1 h	13	33	0.13	0.30	trivial	unlikely	0.491
		POST-+3 h	31	74	0.28	0.56	small	possibly	0.332

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-rps6	RT	PRE-POST	9	105	0.03	0.31	trivial	very unlikely	0.846
		PRE-+1 h	769	885	0.68	0.28	moderate	very likely	< 0.001
		PRE-+3 h	1059	1361	0.71	0.29	moderate	very likely	< 0.001
		POST-+1 h	700	678	0.75	0.28	moderate	very likely	< 0.001
		POST-+3 h	967	1047	0.85	0.31	moderate	very likely	< 0.001
	HIT+RT	PRE-POST	16	48	0.06	0.15	trivial	most unlikely	0.477
		PRE-+1 h	568	827	0.63	0.35	moderate	likely	0.007
		PRE-+3 h	357	420	0.44	0.24	small	possibly	0.007
		POST-+1 h	475	572	0.66	0.33	moderate	likely	0.005
		POST-+3 h	294	319	0.51	0.28	small	likely	0.006
	MICT+RT	PRE-POST	7	23	0.05	0.16	trivial	most unlikely	0.711
		PRE-+1 h	673	502	1.44	0.43	large	most likely	0.001
		PRE-+3 h	195	158	0.67	0.31	moderate	likely	0.032
		POST-+1 h	621	420	1.49	0.42	large	most likely	< 0.001
		POST-+3 h	176	200	0.76	0.51	moderate	likely	0.026
Total-rps6	RT	PRE-POST	-2	12	-0.03	0.22	trivial	very unlikely	0.858
		PRE-+1 h	15	21	0.23	0.31	small	unlikely	0.324
		PRE-+3 h	17	29	0.22	0.35	small	unlikely	0.235
		POST-+1 h	17	21	0.28	0.32	small	unlikely	0.136
		POST-+3 h	19	24	0.31	0.36	small	possibly	0.119

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	HIT+RT	PRE-POST	11	16	0.05	0.07	trivial	most unlikely	0.231
		PRE-+1 h	12	17	0.05	0.07	trivial	most unlikely	0.223
		PRE-+3 h	23	31	0.09	0.11	trivial	most unlikely	0.146
		POST-+1 h	1	9	0.00	0.05	trivial	most unlikely	0.881
		POST-+3 h	11	15	0.05	0.07	trivial	most unlikely	0.163
	MICT+RT	PRE-POST	17	26	0.07	0.10	trivial	most unlikely	0.204
		PRE-+1 h	35	28	0.12	0.09	trivial	most unlikely	0.022
		PRE-+3 h	31	26	0.10	0.08	trivial	most unlikely	0.080
		POST-+1 h	15	27	0.07	0.11	trivial	most unlikely	0.236
		POST-+3 h	12	27	0.05	0.11	trivial	most unlikely	0.364
p-4E-BP1	RT	PRE-POST	35	32	0.37	0.28	small	possibly	0.216
		PRE-+1 h	26	55	0.28	0.50	small	possibly	0.450
		PRE-+3 h	22	30	0.21	0.26	small	unlikely	0.505
		POST-+1 h	-7	21	-0.09	0.28	trivial	very unlikely	0.535
		POST-+3 h	-10	20	-0.13	0.26	trivial	very unlikely	0.400
	HIT+RT	PRE-POST	-7	20	-0.12	0.36	trivial	unlikely	0.651
		PRE-+1 h	-6	21	-0.10	0.36	trivial	unlikely	0.692
		PRE-+3 h	11	25	0.15	0.34	trivial	unlikely	0.536
		POST-+1 h	1	27	0.01	0.45	trivial	unlikely	0.956
		POST-+3 h	18	37	0.29	0.53	small	possibly	0.289

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	21	35	0.29	0.42	small	possibly	0.341
		PRE-+1 h	32	36	0.37	0.36	small	possibly	0.187
		PRE-+3 h	59	53	0.56	0.40	small	likely	0.027
		POST-+1 h	9	26	0.13	0.35	trivial	unlikely	0.461
		POST-+3 h	59	53	0.70	0.49	moderate	likely	0.233
Total-4E-BP1	RT	PRE-POST	-3	20	-0.02	0.12	trivial	most unlikely	0.758
		PRE-+1 h	-20	56	-0.11	0.34	trivial	unlikely	0.508
		PRE-+3 h	-27	73	-0.13	0.38	trivial	unlikely	0.488
		POST-+1 h	-17	44	-0.11	0.30	trivial	unlikely	0.474
		POST-+3 h	-24	59	-0.16	0.43	trivial	unlikely	0.449
	HIT+RT	PRE-POST	13	15	0.27	0.28	small	unlikely	0.213
		PRE-+1 h	12	15	0.25	0.28	small	unlikely	0.297
		PRE-+3 h	11	13	0.23	0.25	small	unlikely	0.394
		POST-+1 h	-1	6	-0.02	0.14	trivial	most unlikely	0.770
		POST-+3 h	-2	8	-0.04	0.17	trivial	most unlikely	0.759
	MICT+RT	PRE-POST	8	9	0.11	0.11	trivial	most unlikely	0.137
		PRE-+1 h	10	32	0.12	0.36	trivial	unlikely	0.548
		PRE-+3 h	-3	6	-0.04	0.07	trivial	most unlikely	0.502
		POST-+1 h	2	26	0.02	0.35	trivial	very unlikely	0.896
		POST-+3 h	-11	10	-0.16	0.16	trivial	very unlikely	0.078

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-GSK-3β	RT	PRE-POST	31	47	0.22	0.29	small	unlikely	0.385
		PRE-+1 h	36	68	0.23	0.36	small	unlikely	0.463
		PRE-+3 h	50	92	0.26	0.37	small	possibly	0.332
		POST-+1 h	4	41	0.03	0.31	trivial	very unlikely	0.867
		POST-+3 h	15	53	0.11	0.36	trivial	unlikely	0.542
	HIT+RT	PRE-POST	22	22	0.38	0.35	small	possibly	0.118
		PRE-+1 h	49	24	0.72	0.28	moderate	very likely	0.028
		PRE-+3 h	51	38	0.68	0.41	moderate	likely	0.038
		POST-+1 h	23	19	0.40	0.30	small	possibly	0.059
		POST-+3 h	24	26	0.43	0.41	small	possibly	0.084
	MICT+RT	PRE-POST	23	20	0.37	0.29	small	possibly	0.034
		PRE-+1 h	40	25	0.55	0.29	small	likely	0.015
		PRE-+3 h	31	27	0.41	0.31	small	possibly	0.031
		POST-+1 h	14	13	0.23	0.21	small	unlikely	0.095
		POST-+3 h	7	18	0.11	0.31	trivial	unlikely	0.441
Total GSK-3β	RT	PRE-POST	-1	25	-0.02	0.33	trivial	very unlikely	0.923
		PRE-+1 h	-13	24	-0.17	0.34	trivial	unlikely	0.481
		PRE-+3 h	-20	18	-0.29	0.29	small	unlikely	0.276
		POST-+1 h	-11	8	-0.16	0.11	trivial	most unlikely	0.176
		POST-+3 h	-19	10	-0.28	0.16	small	unlikely	0.115

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	HIT+RT	PRE-POST	1	12	0.03	0.26	trivial	very unlikely	0.866
		PRE-+1 h	-10	6	-0.23	0.15	small	very unlikely	0.448
		PRE-+3 h	-25	13	-0.67	0.41	moderate	likely	0.052
		POST-+1 h	-11	10	-0.26	0.25	small	unlikely	0.310
		POST-+3 h	-25	14	-0.68	0.44	moderate	likely	0.029
	MICT+RT	PRE-POST	-3	30	-0.02	0.24	trivial	very unlikely	0.862
		PRE-+1 h	-8	24	-0.07	0.20	trivial	very unlikely	0.668
		PRE-+3 h	-18	39	-0.15	0.36	trivial	unlikely	0.680
		POST-+1 h	-5	26	-0.05	0.22	trivial	very unlikely	0.689
		POST-+3 h	-15	27	-0.13	0.25	trivial	very unlikely	0.641
p-eEF2	RT	PRE-POST	21	45	0.13	0.25	trivial	very unlikely	0.527
		PRE-+1 h	-13	53	-0.09	0.37	trivial	unlikely	0.741
		PRE-+3 h	-4	69	-0.02	0.37	trivial	very unlikely	0.928
		POST-+1 h	-28	23	-0.23	0.22	small	unlikely	0.100
		POST-+3 h	-21	42	-0.16	0.35	trivial	unlikely	0.407
	HIT+RT	PRE-POST	11	21	0.13	0.23	trivial	very unlikely	0.496
		PRE-+1 h	-37	8	-0.58	0.15	small	very likely	0.030
		PRE-+3 h	-28	24	-0.39	0.38	small	possibly	0.195
		POST-+1 h	-43	11	-0.71	0.25	moderate	very likely	0.001
		POST-+3 h	-35	27	-0.54	0.50	small	possibly	0.068

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	10	18	0.09	0.17	trivial	very unlikely	0.310
		PRE-+1 h	-35	36	-0.40	0.48	small	possibly	0.149
		PRE-+3 h	-31	12	-0.30	0.14	small	unlikely	0.003
		POST-+1 h	-41	30	-0.53	0.50	small	possibly	0.067
		POST-+3 h	-37	14	-0.47	0.22	small	possibly	0.004
p-ACC	RT	PRE-POST	47	76	0.24	0.30	small	unlikely	0.153
		PRE-+1 h	-6	68	-0.03	0.37	trivial	unlikely	0.854
		PRE-+3 h	-19	40	-0.11	0.24	trivial	very unlikely	0.422
		POST-+1 h	-36	22	-0.28	0.20	small	unlikely	0.026
		POST-+3 h	-45	20	-0.37	0.22	small	possibly	0.012
	HIT+RT	PRE-POST	14	21	0.10	0.14	trivial	most unlikely	0.373
		PRE-+1 h	44	68	0.26	0.33	small	unlikely	0.321
		PRE-+3 h	69	85	0.35	0.32	small	possibly	0.143
		POST-+1 h	27	41	0.18	0.24	trivial	unlikely	0.319
		POST-+3 h	48	82	0.30	0.40	small	possibly	0.196
	MICT+RT	PRE-POST	15	32	0.12	0.25	trivial	very unlikely	0.380
		PRE-+1 h	-38	24	-0.42	0.33	small	possibly	0.089
		PRE-+3 h	-12	22	-0.12	0.23	trivial	very unlikely	0.708
		POST-+1 h	-46	20	-0.56	0.33	small	likely	0.016
		POST-+3 h	-23	26	-0.24	0.29	small	unlikely	0.371

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-AMPK	RT	PRE-POST	29	106	0.15	0.44	trivial	unlikely	0.586
		PRE-+1 h	129	141	0.44	0.31	small	possibly	0.035
		PRE-+3 h	98	189	0.34	0.42	small	possibly	0.082
		POST-+1 h	78	72	0.34	0.23	small	possibly	0.031
		POST-+3 h	166	249	0.58	0.49	small	possibly	0.184
	HIT+RT	PRE-POST	5	61	0.04	0.41	trivial	unlikely	0.860
		PRE-+1 h	19	69	0.12	0.38	trivial	unlikely	0.578
		PRE-+3 h	-2	42	-0.02	0.28	trivial	very unlikely	0.935
		POST-+1 h	13	93	0.09	0.56	trivial	unlikely	0.747
		POST-+3 h	-7	28	-0.06	0.22	trivial	very unlikely	0.647
	MICT+RT	PRE-POST	42	48	0.32	0.31	small	possibly	0.123
		PRE-+1 h	81	54	0.54	0.27	small	likely	0.046
		PRE-+3 h	17	39	0.15	0.30	trivial	unlikely	0.592
		POST-+1 h	28	47	0.23	0.33	small	unlikely	0.220
		POST-+3 h	-17	12	-0.18	0.13	trivial	very unlikely	0.187
Total AMPK	RT	PRE-POST	8	7	0.26	0.20	small	unlikely	0.073
		PRE-+1 h	5	9	0.16	0.24	trivial	unlikely	0.344
		PRE-+3 h	1	13	0.04	0.35	trivial	very unlikely	0.866
		POST-+1 h	-3	9	-0.09	0.30	trivial	very unlikely	0.573
		POST-+3 h	-6	10	-0.21	0.35	small	unlikely	0.296

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	HIT+RT	PRE-POST	32	16	0.63	0.28	moderate	likely	0.091
		PRE-+1 h	43	19	0.80	0.29	moderate	very likely	0.029
		PRE-+3 h	12	23	0.22	0.40	small	unlikely	0.536
		POST-+1 h	9	16	0.19	0.33	trivial	unlikely	0.281
		POST-+3 h	-15	11	-0.37	0.30	small	possibly	0.039
	MICT+RT	PRE-POST	13	19	0.26	0.37	small	unlikely	0.225
		PRE-+1 h	1	12	0.03	0.23	trivial	very unlikely	0.819
		PRE-+3 h	7	19	0.11	0.30	trivial	unlikely	0.448
		POST-+1 h	-10	14	-0.23	0.33	small	unlikely	0.197
		POST-+3 h	-6	9	-0.12	0.19	trivial	very unlikely	0.369
p-TIF-1A	RT	PRE-POST	34	95	0.16	0.37	trivial	unlikely	0.421
		PRE-+1 h	199	151	0.54	0.24	small	likely	0.005
		PRE-+3 h	357	485	0.67	0.41	moderate	likely	0.012
		POST-+1 h	123	79	0.45	0.19	small	possibly	0.002
		POST-+3 h	241	315	0.69	0.46	moderate	likely	0.017
	HIT+RT	PRE-POST	133	102	0.62	0.31	moderate	likely	0.047
		PRE-+1 h	211	75	0.76	0.16	moderate	most likely	0.034
		PRE-+3 h	283	268	0.79	0.38	moderate	very likely	0.006
		POST-+1 h	33	65	0.21	0.34	small	unlikely	0.301
		POST-+3 h	64	80	0.36	0.34	small	possibly	0.108

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	35	54	0.26	0.34	small	unlikely	0.255
		PRE-+1 h	77	83	0.45	0.36	small	possibly	0.075
		PRE-+3 h	16	68	0.11	0.40	trivial	unlikely	0.645
		POST-+1 h	31	103	0.23	0.62	small	possibly	0.434
		POST-+3 h	-13	48	-0.12	0.46	trivial	unlikely	0.571
Total-TIF-1A	RT	PRE-POST	-1	17	-0.02	0.22	trivial	very unlikely	0.866
		PRE-+1 h	-28	25	-0.38	0.39	small	possibly	0.108
		PRE-+3 h	-43	19	-0.56	0.32	small	likely	0.010
		POST-+1 h	-27	18	-0.41	0.32	small	possibly	0.042
		POST-+3 h	-42	19	-0.70	0.42	moderate	likely	0.010
	HIT+RT	PRE-POST	-16	12	-0.17	0.14	trivial	very unlikely	0.047
		PRE-+1 h	-24	17	-0.24	0.20	small	unlikely	0.059
		PRE-+3 h	-42	17	-0.41	0.21	small	possibly	0.038
		POST-+1 h	-9	25	-0.10	0.27	trivial	very unlikely	0.471
		POST-+3 h	-32	28	-0.38	0.40	small	possibly	0.203
	MICT+RT	PRE-POST	-5	23	-0.03	0.14	trivial	most unlikely	0.684
		PRE-+1 h	-59	74	-0.44	0.67	small	possibly	0.197
		PRE-+3 h	-18	24	-0.08	0.12	trivial	most unlikely	0.222
		POST-+1 h	-56	62	-0.47	0.66	small	possibly	0.164
		POST-+3 h	-13	26	-0.08	0.17	trivial	very unlikely	0.335

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
p-UBF	RT	PRE-POST	-3	18	-0.05	0.26	trivial	very unlikely	0.881
		PRE-+1 h	72	40	0.76	0.32	moderate	very likely	0.018
		PRE-+3 h	117	67	0.93	0.37	moderate	very likely	0.013
		POST-+1 h	78	58	0.82	0.45	moderate	likely	0.010
		POST-+3 h	125	72	1.15	0.45	moderate	very likely	0.001
	HIT+RT	PRE-POST	18	20	0.29	0.29	small	unlikely	0.220
		PRE-+1 h	42	25	0.55	0.27	small	likely	0.061
		PRE-+3 h	36	41	0.42	0.40	small	possibly	0.088
		POST-+1 h	20	12	0.32	0.17	small	unlikely	0.022
		POST-+3 h	16	23	0.25	0.34	small	unlikely	0.179
	MICT+RT	PRE-POST	26	34	0.28	0.32	small	unlikely	0.144
		PRE-+1 h	41	39	0.38	0.31	small	possibly	0.079
		PRE-+3 h	2	18	0.02	0.19	trivial	most unlikely	0.825
		POST-+1 h	12	40	0.14	0.42	trivial	unlikely	0.516
		POST-+3 h	-19	14	-0.25	0.21	small	unlikely	0.047
Total-UBF	RT	PRE-POST	1	8	0.01	0.15	trivial	most unlikely	0.869
		PRE-+1 h	-7	14	-0.11	0.23	trivial	very unlikely	0.453
		PRE-+3 h	0	15	0.01	0.20	trivial	most unlikely	0.960
		POST-+1 h	-7	19	-0.13	0.35	trivial	very unlikely	0.479
		POST-+3 h	0	22	-0.01	0.39	trivial	very unlikely	0.976

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	HIT+RT	PRE-POST	9	17	0.15	0.25	trivial	very unlikely	0.291
		PRE-+1 h	17	14	0.23	0.17	small	unlikely	0.031
		PRE-+3 h	20	22	0.26	0.26	small	unlikely	0.079
		POST-+1 h	7	16	0.11	0.24	trivial	very unlikely	0.426
		POST-+3 h	9	22	0.15	0.32	trivial	unlikely	0.400
	MICT+RT	PRE-POST	18	14	0.44	0.31	small	possibly	0.023
		PRE-+1 h	8	21	0.20	0.47	small	unlikely	0.449
		PRE-+3 h	21	14	0.46	0.26	small	possibly	0.105
		POST-+1 h	-8	18	-0.23	0.51	small	possibly	0.378
		POST-+3 h	2	13	0.06	0.34	trivial	unlikely	0.803
Total Cyclin D1	RT	PRE-POST	-1	11	0.00	0.07	trivial	most unlikely	0.914
		PRE-+1 h	-15	12	-0.11	0.09	trivial	most unlikely	0.039
		PRE-+3 h	-22	17	-0.16	0.14	trivial	very unlikely	0.076
		POST-+1 h	-16	12	-0.11	0.09	trivial	most unlikely	0.047
		POST-+3 h	-22	18	-0.16	0.15	trivial	very unlikely	0.073
	HIT+RT	PRE-POST	-11	14	-0.19	0.24	trivial	unlikely	0.418
		PRE-+1 h	-41	12	-0.85	0.32	moderate	very likely	0.001
		PRE-+3 h	-20	9	-0.35	0.19	small	possibly	0.110
		POST-+1 h	-34	7	-0.66	0.16	moderate	very likely	0.008
		POST-+3 h	-10	13	-0.16	0.23	trivial	very unlikely	0.178

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	-17	8	-0.19	0.10	trivial	most unlikely	0.064
		PRE-+1 h	-31	21	-0.38	0.31	small	possibly	0.092
		PRE-+3 h	-2	32	-0.02	0.32	trivial	very unlikely	0.935
		POST-+1 h	-17	28	-0.19	0.34	trivial	unlikely	0.293
		POST-+3 h	18	41	0.17	0.35	trivial	unlikely	0.398

mRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
MuRF1 mRNA	RT	PRE-POST	-21	24	-0.39	0.50	small	possibly	0.169
		POST-+3 h	14	58	0.22	0.80	small	possibly	0.602
		PRE-+3 h	-10	25	-0.18	0.45	trivial	unlikely	0.568
	HIT+RT	PRE-POST	-16	14	-0.21	0.20	small	unlikely	0.256
		POST-+3 h	206	162	1.35	0.61	large	very likely	0.003
		PRE-+3 h	158	116	1.15	0.52	moderate	very likely	0.003
	MICT+RT	PRE-POST	6	29	0.06	0.26	trivial	very unlikely	0.731
		POST-+3 h	22	76	0.20	0.58	small	possibly	0.588
		PRE-+3 h	30	74	0.26	0.54	small	possibly	0.560
Atrogin-1 mRNA	RT	PRE-POST	-15	25	-0.25	0.46	small	possibly	0.491
		POST-+3 h	-44	22	-0.91	0.60	moderate	likely	0.018
		PRE-+3 h	-52	11	-1.16	0.34	moderate	most likely	0.003
	HIT+RT	PRE-POST	-14	20	-0.19	0.28	trivial	unlikely	0.318
		POST-+3 h	6	32	0.07	0.37	trivial	unlikely	0.706
		PRE-+3 h	-9	23	-0.12	0.31	trivial	unlikely	0.483
	MICT+RT	PRE-POST	-12	30	-0.18	0.45	trivial	unlikely	0.642
		POST-+3 h	5	42	0.06	0.54	trivial	unlikely	0.807
		PRE-+3 h	-8	25	-0.12	0.37	trivial	unlikely	0.740

mRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
PGC1α mRNA	RT	PRE-POST	-26	14	-0.48	0.30	small	possibly	0.026
		POST-+3 h	26	48	0.36	0.59	small	possibly	0.287
		PRE-+3 h	-7	28	-0.12	0.46	trivial	unlikely	0.723
	HIT+RT	PRE-POST	-12	14	-0.26	0.32	small	unlikely	0.294
		POST-+3 h	826	349	4.58	0.76	extremely large	most likely	< 0.001
		PRE-+3 h	716	222	4.32	0.55	extremely large	most likely	< 0.001
	MICT+RT	PRE-POST	-45	13	-0.61	0.23	moderate	likely	0.157
		POST-+3 h	590	481	1.97	0.66	large	most likely	0.001
		PRE-+3 h	281	207	1.37	0.53	large	very likely	0.023
Fox-O1 mRNA	RT	PRE-POST	28	17	0.49	0.27	small	possibly	0.051
		POST-+3 h	7	25	0.13	0.45	trivial	unlikely	0.587
		PRE-+3 h	37	23	0.63	0.34	small	likely	0.010
	HIT+RT	PRE-POST	67	60	0.32	0.22	small	possibly	0.504
		POST-+3 h	158	65	0.59	0.16	small	very likely	< 0.001
		PRE-+3 h	330	138	0.92	0.20	moderate	most likely	0.076
	MICT+RT	PRE-POST	35	42	0.36	0.36	small	possibly	0.098
		POST-+3 h	36	49	0.37	0.41	small	possibly	0.294
		PRE-+3 h	84	46	0.73	0.29	moderate	very likely	0.093

mRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
TIF-1A mRNA	RT	PRE-POST	-6	14	-0.14	0.33	trivial	unlikely	0.433
		POST-+3 h	26	12	0.53	0.21	small	likely	0.003
		PRE-+3 h	19	18	0.39	0.33	small	possibly	0.086
	HIT+RT	PRE-POST	-8	12	-0.11	0.17	trivial	very unlikely	0.279
		POST-+3 h	5	33	0.07	0.41	trivial	unlikely	0.753
		PRE-+3 h	-3	33	-0.04	0.44	trivial	very unlikely	0.860
	MICT+RT	PRE-POST	-8	11	-0.16	0.24	trivial	unlikely	0.214
		POST-+3 h	36	35	0.59	0.50	small	likely	0.038
		PRE-+3 h	25	21	0.43	0.32	small	possibly	0.028
UBF mRNA	RT	PRE-POST	-14	13	-0.30	0.30	small	possibly	0.100
		POST-+3 h	-20	28	-0.45	0.69	small	possibly	0.218
		PRE-+3 h	-31	18	-0.75	0.51	moderate	likely	0.025
	HIT+RT	PRE-POST	8	8	0.14	0.15	trivial	very unlikely	0.421
		POST-+3 h	-9	17	-0.17	0.35	trivial	unlikely	0.386
		PRE-+3 h	-2	19	-0.04	0.35	trivial	very unlikely	0.837
	MICT+RT	PRE-POST	4	15	0.10	0.32	trivial	unlikely	0.533
		POST-+3 h	-11	17	-0.27	0.44	small	possibly	0.361
		PRE-+3 h	-7	19	-0.17	0.46	trivial	unlikely	0.599

mRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
POLR1B mRNA	RT	PRE-POST	-26	16	-0.44	0.32	small	possibly	0.026
		POST-+3 h	8	32	0.11	0.42	trivial	very unlikely	0.622
		PRE-+3 h	-20	19	-0.33	0.35	small	possibly	0.087
	HIT+RT	PRE-POST	1	10	0.02	0.15	trivial	most unlikely	0.810
		POST-+3 h	44	42	0.57	0.44	small	likely	0.047
		PRE-+3 h	46	38	0.59	0.40	small	likely	0.023
	MICT+RT	PRE-POST	-5	20	-0.06	0.27	trivial	very unlikely	0.634
		POST-+3 h	48	43	0.51	0.37	small	possibly	0.033
		PRE-+3 h	41	33	0.45	0.30	small	possibly	0.025
Cyclin D1 mRNA	RT	PRE-POST	51	45	0.29	0.21	small	unlikely	0.424
		POST-+3 h	10	41	0.07	0.26	trivial	very unlikely	0.603
		PRE-+3 h	66	68	0.36	0.28	small	possibly	0.364
	HIT+RT	PRE-POST	101	54	0.59	0.22	small	likely	0.001
		POST-+3 h	10	43	0.08	0.32	trivial	unlikely	0.648
		PRE-+3 h	121	115	0.66	0.42	moderate	likely	0.014
	MICT+RT	PRE-POST	36	67	0.29	0.44	small	possibly	0.292
		POST-+3 h	4	44	0.04	0.38	trivial	unlikely	0.833
		PRE-+3 h	42	63	0.33	0.41	small	possibly	0.217

rRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
Total RNA	RT	PRE-POST	-25	11	-0.19	0.09	trivial	most unlikely	0.030
	HIT+RT	PRE-POST	47	15	0.39	0.10	small	possibly	0.023
	MICT+RT	PRE-POST	27	26	0.08	0.07	trivial	most unlikely	0.060
45S pre-rRNA	RT	PRE-POST	-32	22	-0.40	0.33	small	possibly	0.138
		POST-+3 h	12	42	0.12	0.39	trivial	very unlikely	0.539
		PRE-+3 h	-23	34	-0.27	0.45	small	possibly	0.314
	HIT+RT	PRE-POST	8	26	0.07	0.21	trivial	very unlikely	0.514
		POST-+3 h	69	107	0.48	0.54	small	possibly	0.110
		PRE-+3 h	83	116	0.55	0.54	small	possibly	0.071
	MICT+RT	PRE-POST	20	24	0.21	0.22	small	unlikely	0.099
		POST-+3 h	43	62	0.40	0.47	small	possibly	0.121
		PRE-+3 h	71	80	0.61	0.51	moderate	likely	0.041
5.8S rRNA	RT	PRE-POST	-51	16	-0.69	0.31	moderate	likely	0.017
		POST-+3 h	22	63	0.20	0.48	small	unlikely	0.421
		PRE-+3 h	-40	29	-0.49	0.44	small	possibly	0.099
	HIT+RT	PRE-POST	10	20	0.08	0.15	trivial	most unlikely	0.469
		POST-+3 h	39	87	0.27	0.47	small	possibly	0.280
		PRE-+3 h	54	108	0.35	0.52	small	possibly	0.244

rRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	8	36	0.05	0.24	trivial	very unlikely	0.632
		POST-+3 h	46	73	0.27	0.35	small	unlikely	0.200
		PRE-+3 h	57	97	0.33	0.42	small	possibly	0.194
5.8S rRNA (span)	RT	PRE-POST	-36	15	-0.51	0.27	small	likely	0.027
		POST-+3 h	2	26	0.02	0.29	trivial	very unlikely	0.923
		PRE-+3 h	-35	19	-0.49	0.33	small	possibly	0.017
	HIT+RT	PRE-POST	35	35	0.29	0.24	small	unlikely	0.244
		POST-+3 h	54	74	0.41	0.44	small	possibly	0.127
		PRE-+3 h	95	100	0.63	0.47	moderate	likely	0.033
	MICT+RT	PRE-POST	-1	47	-0.01	0.47	trivial	unlikely	0.962
		POST-+3 h	29	61	0.26	0.46	small	possibly	0.269
		PRE-+3 h	27	59	0.25	0.45	small	possibly	0.330
18S rRNA	RT	PRE-POST	112	59	0.34	0.12	small	unlikely	0.509
		POST-+3 h	-8	41	-0.04	0.20	trivial	very unlikely	0.732
		PRE-+3 h	96	40	0.30	0.09	small	very unlikely	0.525
	HIT+RT	PRE-POST	3	13	0.04	0.17	trivial	most unlikely	0.762
		POST-+3 h	28	54	0.34	0.55	small	possibly	0.243
		PRE-+3 h	33	44	0.38	0.44	small	possibly	0.142

rRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	-22	17	-0.30	0.26	small	unlikely	0.429
		POST-+3 h	-9	37	-0.11	0.47	trivial	unlikely	0.635
		PRE-+3 h	-29	19	-0.41	0.31	small	possibly	0.270
18S rRNA (span)	RT	PRE-POST	-35	29	-0.30	0.30	small	possibly	0.078
		POST-+3 h	29	90	0.18	0.45	trivial	unlikely	0.446
		PRE-+3 h	-16	40	-0.12	0.32	trivial	unlikely	0.465
	HIT+RT	PRE-POST	28	85	0.11	0.29	trivial	unlikely	0.509
		POST-+3 h	49	106	0.18	0.30	trivial	unlikely	0.255
		PRE-+3 h	90	186	0.30	0.40	small	possibly	0.222
	MICT+RT	PRE-POST	4	22	0.02	0.09	trivial	most unlikely	0.773
		POST-+3 h	63	48	0.21	0.12	small	very unlikely	0.029
		PRE-+3 h	69	75	0.22	0.18	small	unlikely	0.106
28S rRNA	RT	PRE-POST	-33	15	-0.49	0.28	small	possibly	0.037
		POST-+3 h	3	33	0.03	0.39	trivial	unlikely	0.867
		PRE-+3 h	-31	22	-0.46	0.39	small	possibly	0.052
	HIT+RT	PRE-POST	16	14	0.16	0.13	trivial	most unlikely	0.097
		POST-+3 h	17	59	0.16	0.51	trivial	unlikely	0.530
		PRE-+3 h	36	76	0.32	0.56	small	possibly	0.287

rRNA target	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
	MICT+RT	PRE-POST	10	20	0.13	0.25	trivial	very unlikely	0.380
		POST-+3 h	4	38	0.05	0.49	trivial	unlikely	0.826
		PRE-+3 h	14	46	0.18	0.53	trivial	unlikely	0.525
28S rRNA (span)	RT	PRE-POST	-19	21	-0.22	0.27	small	unlikely	0.163
		POST-+3 h	1	56	0.01	0.55	trivial	unlikely	0.963
		PRE-+3 h	-18	34	-0.20	0.42	small	unlikely	0.362
	HIT+RT	PRE-POST	27	40	0.26	0.32	small	unlikely	0.251
		POST-+3 h	-21	26	-0.24	0.34	small	unlikely	0.186
		PRE-+3 h	1	43	0.01	0.44	trivial	unlikely	0.970
	MICT+RT	PRE-POST	-15	15	-0.20	0.21	small	unlikely	0.229
		POST-+3 h	44	58	0.44	0.48	small	possibly	0.093
		PRE-+3 h	22	59	0.24	0.56	small	possibly	0.394

Measure	Group	Comparison	Mean difference		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial	*P* value
			% difference	±90% CL	ES (*d*)	±90% CL			
Type I fibre CSA	RT	PRE-POST	15	13	0.10	0.08	trivial	most unlikely	0.035
	HIT+RT	PRE-POST	-23	19	-0.09	0.08	trivial	most unlikely	0.135
	MICT+RT	PRE-POST	0.4	17	0.00	-0.14	trivial	most unlikely	0.989
Type II fibre CSA	RT	PRE-POST	19	27	0.09	0.12	trivial	most unlikely	0.139
	HIT+RT	PRE-POST	0.4	24	0.00	0.08	trivial	most unlikely	0.974
	MICT+RT	PRE-POST	16	14	0.19	0.16	trivial	very likely	0.344

Appendix P: Between-group comparison data for Study 2 (Chapters 4 and 5)[13]

[13] Magnitude-based inference data were calculated using 0.20 as the smallest worthwhile effect for all performance, body composition, and nutritional measures, whereas 0.40 was used for all molecular data as per comparisons for Study 1 (Chapter 3)

Measure	Group comparison	Mean difference in PRE-POST change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
		% difference	±90% CL	ES (*d*)	±90% CL		
Maximal strength							
1RM leg press	HIT+RT vs. RT	-7.4	8.7	-0.40	0.40	small	likely
	MICT+RT vs. RT	-8.2	9.9	-0.60	0.45	moderate	likely
	HIT+RT vs. MICT+RT	0.9	8.1	0.03	0.30	trivial	unlikely
1RM bench press	HIT+RT vs. RT	-1.0	4.7	-0.04	0.22	trivial	possibly
	MICT+RT vs. RT	-4.7	6.1	-0.15	0.20	trivial	possibly
	HIT+RT vs. MICT+RT	-0.9	8.1	-0.03	0.19	trivial	unlikely
Counter-movement jump (CMJ) variables							
Peak CMJ force	HIT+RT vs. RT	-6.8	4.5	-0.41	0.28	small	likely
	MICT+RT vs. RT	-9.9	11.2	-0.54	0.65	small	likely
	HIT+RT vs. MICT+RT	-5.0	12.1	-0.33	0.19	small	possibly
Peak CMJ power	HIT+RT vs. RT	-5.1	7.3	-0.38	0.56	small	possibly
	MICT+RT vs. RT	-3.5	8.7	-0.21	0.54	small	possibly
	HIT+RT vs. MICT+RT	1.7	8.4	0.10	0.48	trivial	possibly
Peak CMJ velocity	HIT+RT vs. RT	-6.4	9.1	-0.32	0.46	small	possibly
	MICT+RT vs. RT	-3.3	9.3	-0.16	0.46	trivial	possibly
	HIT+RT vs. MICT+RT	1.4	5.6	0.15	0.61	trivial	possibly

Measure	Group comparison	Mean difference in PRE-POST change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
		% difference	±90% CL	ES (*d*)	±90% CL		
Peak CMJ displacement	HIT+RT vs. RT	-1.6	11.6	-0.06	0.43	trivial	possibly
	MICT+RT vs. RT	-7.3	10.4	-0.28	0.41	small	possibly
	HIT+RT vs. MICT+RT	5.8	9.8	0.42	0.74	small	possibly
Body composition							
Lean mass (lower)	HIT+RT vs. RT	-2.2	2.8	-0.18	0.23	trivial	possibly
	MICT+RT vs. RT	-0.5	3.5	-0.05	0.35	trivial	unlikely
	HIT+RT vs. MICT+RT	1.7	3.1	0.16	0.28	trivial	possibly
Lean mass (upper)	HIT+RT vs. RT	1.0	2.5	0.08	0.19	trivial	unlikely
	MICT+RT vs. RT	1.4	3.5	0.10	0.26	trivial	possibly
	HIT+RT vs. MICT+RT	0.4	3.5	0.04	0.34	trivial	unlikely
Lean mass (total)	HIT+RT vs. RT	0.1	1.9	0.01	0.15	trivial	very unlikely
	MICT+RT vs. RT	0.8	3.1	0.06	0.27	trivial	unlikely
	HIT+RT vs. MICT+RT	0.8	2.9	0.08	0.29	trivial	unlikely
Body fat %	HIT+RT vs. RT	2.0	8.0	0.05	0.20	trivial	unlikely
	MICT+RT vs. RT	1.9	7.8	0.06	0.24	trivial	unlikely
	HIT+RT vs. MICT+RT	3.8	6.8	0.12	0.23	trivial	possibly

Measure	Group comparison	Mean difference in PRE-POST change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
		% difference	±90% CL	ES (*d*)	±90% CL		
Aerobic capacity							
Absolute $\dot{V}O_{2peak}$	HIT+RT vs. RT	5.9	9.4	0.26	0.40	small	possibly
	MICT+RT vs. RT	6.8	9.3	0.27	0.35	small	possibly
	HIT+RT vs. MICT+RT	0.8	8.1	0.04	0.38	trivial	unlikely
Relative $\dot{V}O_{2peak}$	HIT+RT vs. RT	6.3	10.2	0.19	0.30	trivial	possibly
	MICT+RT vs. RT	7.4	9.4	0.24	0.29	small	possibly
	HIT+RT vs. MICT+RT	1.0	8.3	0.04	0.29	trivial	unlikely
Lactate threshold	HIT+RT vs. RT	0.9	10.2	0.02	0.24	trivial	unlikely
	MICT+RT vs. RT	4.9	14.6	0.12	0.35	trivial	possibly
	HIT+RT vs. MICT+RT	4.0	13.7	0.11	0.37	trivial	possibly
Peak aerobic power	HIT+RT vs. RT	11.3	8.1	0.35	0.24	small	likely
	MICT+RT vs. RT	7.3	7.8	0.24	0.25	small	possibly
	HIT+RT vs. MICT+RT	-3.6	5.9	-0.16	0.26	trivial	possibly

Measure	Group comparison	Mean difference in PRE-POST change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
		% difference	±90% CL	ES (*d*)	±90% CL		
Average daily nutritional intake							
Average daily energy intake	HIT+RT vs. RT	14.2	20.5	0.62	0.84	moderate	likely
	MICT+RT vs. RT	3.4	17.9	0.17	0.86	trivial	possibly
	HIT+RT vs. MICT+RT	9.5	15.4	0.51	0.86	small	possibly
Average daily protein intake	HIT+RT vs. RT	7.0	20.6	0.29	0.84	small	possibly
	MICT+RT vs. RT	-0.9	16.4	-0.05	0.86	trivial	possibly
	HIT+RT vs. MICT+RT	-7.3	16.1	-0.38	0.85	small	possibly
Average daily carbohydrate intake	HIT+RT vs. RT	0.9	13.8	0.06	0.83	trivial	possibly
	MICT+RT vs. RT	13.4	21.8	0.58	0.88	small	likely
	HIT+RT vs. MICT+RT	-12.3	20.5	-0.57	0.89	small	likely
Average daily fat intake	HIT+RT vs. RT	28.2	34.7	0.77	0.84	moderate	likely
	MICT+RT vs. RT	3.9	20.0	0.16	0.86	trivial	possibly
	HIT+RT vs. MICT+RT	25.0	18.2	1.02	0.85	moderate	likely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
p-mTOR	HIT+RT vs. RT	PRE-POST	7	71	0.07	0.60	trivial	unlikely
		PRE-+1 h	-37	45	-0.34	0.49	small	possibly
		PRE-+3 h	-46	41	-0.48	0.54	small	possibly
		POST-+1 h	-36	47	-0.44	0.66	small	possibly
		POST-+3 h	-29	70	-0.33	0.84	small	possibly
	MICT+RT vs. RT	PRE-POST	-26	68	-0.29	0.80	small	possibly
		PRE-+1 h	-15	88	-0.11	0.62	trivial	unlikely
		PRE-+3 h	-64	36	-0.64	0.55	moderate	likely
		POST-+1 h	-14	90	-0.15	0.89	trivial	possibly
		POST-+3 h	-36	61	-0.44	0.83	small	possibly
	HIT+RT vs. MICT+RT	PRE-POST	-31	53	-0.46	0.88	small	possibly
		PRE-+1 h	24	118	0.18	0.70	trivial	possibly
		PRE-+3 h	-53	43	-0.54	0.58	small	possibly
		POST-+1 h	35	136	0.38	1.11	small	possibly
		POST-+3 h	-10	41	-0.13	0.56	trivial	unlikely
Total mTOR	HIT+RT vs. RT	PRE-POST	1	30	0.03	0.61	trivial	unlikely
		PRE-+1 h	-27	21	-0.51	0.47	small	possibly
		PRE-+3 h	-25	32	-0.36	0.51	small	possibly
		POST-+1 h	-23	20	-0.55	0.54	small	possibly
		POST-+3 h	-31	26	-0.77	0.76	moderate	likely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	MICT+RT vs. RT	PRE-POST	23	44	0.31	0.54	small	possibly
		PRE-+1 h	-25	28	-0.35	0.46	small	possibly
		PRE-+3 h	-27	37	-0.38	0.59	small	possibly
		POST-+1 h	-21	28	-0.36	0.54	small	possibly
		POST-+3 h	-31	30	-0.57	0.65	small	possibly
	HIT+RT vs. MICT+RT	PRE-POST	21	35	0.43	0.64	small	possibly
		PRE-+1 h	-3	34	-0.05	0.55	trivial	unlikely
		PRE-+3 h	4	47	0.06	0.61	trivial	unlikely
		POST-+1 h	3	35	0.07	0.75	trivial	unlikely
		POST-+3 h	1	41	0.01	0.89	trivial	unlikely
p-p70S6K1	HIT+RT vs. RT	PRE-POST	33	97	0.38	0.91	small	possibly
		PRE-+1 h	-58	22	-0.98	0.56	moderate	very likely
		PRE-+3 h	-67	30	-1.02	0.75	moderate	likely
		POST-+1 h	-38	36	-0.65	0.74	moderate	possibly
		POST-+3 h	-47	50	-0.86	1.13	moderate	likely
	MICT+RT vs. RT	PRE-POST	-13	60	-0.17	0.82	trivial	possibly
		PRE-+1 h	-50	27	-0.89	0.67	moderate	likely
		PRE-+3 h	-74	20	-1.53	0.80	large	very likely
		POST-+1 h	-27	44	-0.40	0.73	small	possibly
		POST-+3 h	-50	46	-0.88	1.05	moderate	likely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	-34	37	-0.65	0.83	moderate	possibly
		PRE-+1 h	-39	51	-0.65	1.00	moderate	possibly
		PRE-+3 h	-63	24	-1.16	0.72	moderate	very likely
		POST-+1 h	19	77	0.27	0.94	small	possibly
		POST-+3 h	-5	44	-0.08	0.69	trivial	unlikely
Total-p70S6K1	HIT+RT vs. RT	PRE-POST	7	19	0.12	0.31	trivial	unlikely
		PRE-+1 h	-6	16	-0.08	0.23	trivial	very unlikely
		PRE-+3 h	18	38	0.20	0.38	small	unlikely
		POST-+1 h	-3	19	-0.05	0.34	trivial	very unlikely
		POST-+3 h	3	31	0.05	0.52	trivial	unlikely
	MICT+RT vs. RT	PRE-POST	-22	38	-0.26	0.50	small	possibly
		PRE-+1 h	25	44	0.20	0.30	small	unlikely
		PRE-+3 h	41	52	0.35	0.38	small	possibly
		POST-+1 h	29	46	0.27	0.36	small	possibly
		POST-+3 h	64	93	0.52	0.57	small	possibly
	HIT+RT vs. MICT+RT	PRE-POST	-27	36	-0.47	0.71	small	possibly
		PRE-+1 h	21	44	0.25	0.46	small	possibly
		PRE-+3 h	28	34	0.42	0.45	small	possibly
		POST-+1 h	33	45	0.43	0.50	small	possibly
		POST-+3 h	59	86	0.70	0.78	moderate	likely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
p-rps6	HIT+RT vs. RT	PRE-POST	7	99	0.04	0.48	trivial	unlikely
		PRE-+1 h	-34	86	-0.15	0.40	small	unlikely
		PRE-+3 h	-94	8	-0.95	0.39	moderate	very likely
		POST-+1 h	-28	85	-0.19	0.58	small	possibly
		POST-+3 h	-63	41	-0.57	0.56	small	possibly
	MICT+RT vs. RT	PRE-POST	-1	91	-0.01	0.44	trivial	unlikely
		PRE-+1 h	-17	84	-0.07	0.35	trivial	unlikely
		PRE-+3 h	-97	4	-1.30	0.42	large	most likely
		POST-+1 h	-10	79	-0.06	0.42	trivial	unlikely
		POST-+3 h	-74	29	-0.72	0.51	moderate	likely
	HIT+RT vs. MICT+RT	PRE-POST	-8	46	-0.06	0.38	trivial	unlikely
		PRE-+1 h	8	129	0.03	0.46	trivial	unlikely
		PRE-+3 h	-92	10	-1.01	0.41	moderate	very likely
		POST-+1 h	25	128	0.18	0.72	trivial	possibly
		POST-+3 h	-30	70	-0.29	0.70	small	possibly
Total-rps6	HIT+RT vs. RT	PRE-POST	13	24	0.09	0.15	trivial	most unlikely
		PRE-+1 h	-12	23	-0.07	0.14	trivial	most unlikely
		PRE-+3 h	-6	24	-0.03	0.13	trivial	most unlikely
		POST-+1 h	-14	16	-0.11	0.13	trivial	most unlikely
		POST-+3 h	-7	20	-0.05	0.15	trivial	most unlikely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	MICT+RT vs. RT	PRE-POST	18	29	0.11	0.15	trivial	most unlikely
		PRE-+1 h	1	31	0.00	0.15	trivial	most unlikely
		PRE-+3 h	-17	23	-0.08	0.12	trivial	most unlikely
		POST-+1 h	-1	25	-0.01	0.16	trivial	most unlikely
		POST-+3 h	-6	26	-0.04	0.17	trivial	most unlikely
	HIT+RT vs. MICT+RT	PRE-POST	5	26	0.03	0.17	trivial	most unlikely
		PRE-+1 h	3	26	0.02	0.14	trivial	most unlikely
		PRE-+3 h	-21	22	-0.11	0.13	trivial	most unlikely
		POST-+1 h	15	26	0.09	0.15	trivial	most unlikely
		POST-+3 h	1	25	0.01	0.17	trivial	most unlikely
p-4E-BP1	HIT+RT vs. RT	PRE-POST	-31	34	-0.52	0.67	moderate	possibly
		PRE-+1 h	-20	49	-0.24	0.61	small	possibly
		PRE-+3 h	-3	57	-0.04	0.59	trivial	unlikely
		POST-+1 h	8	32	0.11	0.41	trivial	unlikely
		POST-+3 h	32	45	0.38	0.47	small	possibly
	MICT+RT vs. RT	PRE-POST	-10	48	-0.19	0.90	trivial	possibly
		PRE-+1 h	-14	52	-0.20	0.77	small	possibly
		PRE-+3 h	-1	63	-0.01	0.77	trivial	unlikely
		POST-+1 h	17	32	0.27	0.47	small	possibly
		POST-+3 h	46	66	0.66	0.76	moderate	possibly

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	30	57	0.53	0.87	small	possibly
		PRE-+1 h	16	38	0.22	0.48	small	possibly
		PRE-+3 h	9	47	0.11	0.53	trivial	unlikely
		POST-+1 h	8	32	0.16	0.60	trivial	unlikely
		POST-+3 h	11	52	0.22	0.93	small	possibly
Total-4E-BP1	HIT+RT vs. RT	PRE-POST	17	29	0.27	0.42	small	possibly
		PRE-+1 h	23	78	0.19	0.53	trivial	unlikely
		PRE-+3 h	35	120	0.19	0.50	trivial	unlikely
		POST-+1 h	19	57	0.30	0.79	small	possibly
		POST-+3 h	29	91	0.44	1.12	small	possibly
	MICT+RT vs. RT	PRE-POST	12	24	0.22	0.42	small	unlikely
		PRE-+1 h	27	83	0.23	0.59	small	possibly
		PRE-+3 h	19	109	0.12	0.54	trivial	unlikely
		POST-+1 h	22	63	0.40	0.98	small	possibly
		POST-+3 h	17	83	0.32	1.30	small	possibly
	HIT+RT vs. MICT+RT	PRE-POST	-4	18	-0.08	0.33	trivial	unlikely
		PRE-+1 h	-9	27	-0.14	0.43	trivial	unlikely
		PRE-+3 h	-21	25	-0.32	0.42	small	possibly
		POST-+1 h	3	27	0.05	0.45	trivial	unlikely
		POST-+3 h	-9	12	-0.17	0.24	trivial	unlikely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
p-GSK-3β	HIT+RT vs. RT	PRE-POST	-7	57	-0.10	0.82	trivial	possibly
		PRE-+1 h	-10	76	-0.11	0.80	trivial	possibly
		PRE-+3 h	-33	57	-0.33	0.64	small	possibly
		POST-+1 h	18	49	0.24	0.57	small	possibly
		POST-+3 h	8	49	0.11	0.62	trivial	unlikely
	MICT+RT vs. RT	PRE-POST	-6	56	-0.07	0.61	trivial	unlikely
		PRE-+1 h	-16	69	-0.14	0.61	trivial	unlikely
		PRE-+3 h	-38	52	-0.33	0.54	small	possibly
		POST-+1 h	10	44	0.10	0.42	trivial	unlikely
		POST-+3 h	-7	41	-0.08	0.46	trivial	unlikely
	HIT+RT vs. MICT+RT	PRE-POST	1	24	0.02	0.51	trivial	unlikely
		PRE-+1 h	-24	23	-0.50	0.54	small	possibly
		PRE-+3 h	-38	23	-0.80	0.60	moderate	likely
		POST-+1 h	-7	19	-0.16	0.42	trivial	unlikely
		POST-+3 h	-14	21	-0.33	0.50	small	possibly
Total GSK-3β	HIT+RT vs. RT	PRE-POST	2	28	0.04	0.47	trivial	unlikely
		PRE-+1 h	2	39	0.04	0.62	trivial	unlikely
		PRE-+3 h	4	40	0.08	0.67	trivial	unlikely
		POST-+1 h	1	23	0.02	0.40	trivial	unlikely
		POST-+3 h	-8	26	-0.14	0.50	trivial	unlikely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	MICT+RT vs. RT	PRE-POST	-2	38	-0.02	0.41	trivial	unlikely
		PRE-+1 h	8	44	0.08	0.39	trivial	unlikely
		PRE-+3 h	12	80	0.12	0.68	trivial	unlikely
		POST-+1 h	7	30	0.07	0.30	trivial	very unlikely
		POST-+3 h	5	77	0.05	0.73	trivial	unlikely
	HIT+RT vs. MICT+RT	PRE-POST	-4	33	-0.05	0.42	trivial	unlikely
		PRE-+1 h	5	34	0.05	0.38	trivial	unlikely
		PRE-+3 h	19	80	0.21	0.78	small	possibly
		POST-+1 h	6	32	0.07	0.37	trivial	unlikely
		POST-+3 h	14	83	0.16	0.84	trivial	possibly
p-eEF2	HIT+RT vs. RT	PRE-POST	-8	56	-0.11	0.73	trivial	unlikely
		PRE-+1 h	-35	58	-0.47	0.89	small	possibly
		PRE-+3 h	19	122	0.15	0.77	trivial	possibly
		POST-+1 h	-21	29	-0.31	0.47	small	possibly
		POST-+3 h	-19	50	-0.26	0.75	small	possibly
	MICT+RT vs. RT	PRE-POST	-9	52	-0.12	0.73	trivial	possibly
		PRE-+1 h	-32	65	-0.37	0.80	small	possibly
		PRE-+3 h	12	117	0.08	0.69	trivial	unlikely
		POST-+1 h	-18	45	-0.27	0.70	small	possibly
		POST-+3 h	-21	43	-0.31	0.68	small	possibly

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	-1	30	-0.01	0.46	trivial	unlikely
		PRE-+1 h	-6	52	-0.08	0.68	trivial	unlikely
		PRE-+3 h	49	86	0.46	0.63	small	possibly
		POST-+1 h	4	52	0.06	0.75	trivial	unlikely
		POST-+3 h	-3	41	-0.04	0.64	trivial	unlikely
p-ACC	HIT+RT vs. RT	PRE-POST	-23	40	-0.24	0.47	small	possibly
		PRE-+1 h	35	103	0.21	0.49	small	unlikely
		PRE-+3 h	44	115	0.22	0.44	small	unlikely
		POST-+1 h	99	100	0.65	0.46	moderate	likely
		POST-+3 h	169	168	0.94	0.56	moderate	likely
	MICT+RT vs. RT	PRE-POST	-22	41	-0.23	0.47	small	possibly
		PRE-+1 h	-43	42	-0.39	0.48	small	possibly
		PRE-+3 h	75	106	0.40	0.40	small	possibly
		POST-+1 h	-16	38	-0.16	0.41	trivial	unlikely
		POST-+3 h	39	87	0.30	0.55	small	possibly
	HIT+RT vs. MICT+RT	PRE-POST	1	37	0.01	0.34	trivial	very unlikely
		PRE-+1 h	-63	29	-0.82	0.59	moderate	likely
		PRE-+3 h	-16	62	-0.14	0.56	trivial	unlikely
		POST-+1 h	-58	23	-0.82	0.49	moderate	likely
		POST-+3 h	-48	39	-0.63	0.67	moderate	possibly

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
p-AMPK	HIT+RT vs. RT	PRE-POST	-18	89	-0.18	0.84	trivial	possibly
		PRE-+1 h	-51	47	-0.49	0.58	small	possibly
		PRE-+3 h	-68	39	-0.73	0.66	moderate	likely
		POST-+1 h	-37	52	-0.41	0.68	small	possibly
		POST-+3 h	-54	49	-0.69	0.83	moderate	possibly
	MICT+RT vs. RT	PRE-POST	10	110	0.09	0.79	trivial	unlikely
		PRE-+1 h	-44	40	-0.45	0.51	small	possibly
		PRE-+3 h	-75	26	-1.02	0.67	moderate	likely
		POST-+1 h	-28	37	-0.30	0.45	small	possibly
		POST-+3 h	-59	44	-0.79	0.83	moderate	likely
	HIT+RT vs. MICT+RT	PRE-POST	34	89	0.28	0.60	small	possibly
		PRE-+1 h	7	71	0.06	0.49	trivial	unlikely
		PRE-+3 h	-34	41	-0.34	0.48	small	possibly
		POST-+1 h	13	90	0.12	0.70	trivial	unlikely
		POST-+3 h	-11	33	-0.11	0.35	trivial	unlikely
Total AMPK	HIT+RT vs. RT	PRE-POST	22	33	0.53	0.74	small	possibly
		PRE-+1 h	3	16	0.06	0.31	trivial	very unlikely
		PRE-+3 h	-23	15	-0.46	0.35	small	possibly
		POST-+1 h	12	17	0.30	0.41	small	possibly
		POST-+3 h	-9	14	-0.26	0.42	small	possibly

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	MICT+RT vs. RT	PRE-POST	4	19	0.13	0.57	trivial	unlikely
		PRE-+1 h	-15	14	-0.42	0.43	small	possibly
		PRE-+3 h	4	18	0.08	0.38	trivial	unlikely
		POST-+1 h	-8	15	-0.25	0.50	small	possibly
		POST-+3 h	1	15	0.03	0.47	trivial	unlikely
	HIT+RT vs. MICT+RT	PRE-POST	-14	26	-0.51	0.99	small	possibly
		PRE-+1 h	-37	17	-1.18	0.69	moderate	very likely
		PRE-+3 h	-6	33	-0.13	0.70	trivial	unlikely
		POST-+1 h	-17	15	-0.63	0.60	moderate	possibly
		POST-+3 h	11	17	0.35	0.51	small	possibly
p-TIF-1A	HIT+RT vs. RT	PRE-POST	74	171	0.58	0.91	small	possibly
		PRE-+1 h	-55	32	-0.74	0.61	moderate	likely
		PRE-+3 h	-73	39	-0.80	0.71	moderate	likely
		POST-+1 h	-40	35	-0.54	0.57	small	possibly
		POST-+3 h	-52	46	-0.76	0.89	moderate	likely
	MICT+RT vs. RT	PRE-POST	0	82	0.00	0.71	trivial	unlikely
		PRE-+1 h	-56	36	-0.68	0.63	moderate	likely
		PRE-+3 h	-86	16	-1.21	0.59	large	very likely
		POST-+1 h	-41	43	-0.51	0.65	small	possibly
		POST-+3 h	-75	24	-1.31	0.80	large	very likely

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	-42	48	-0.59	0.82	moderate	possibly
		PRE-+1 h	-58	47	-0.90	1.01	moderate	likely
		PRE-+3 h	-83	15	-1.34	0.60	large	very likely
		POST-+1 h	-2	80	-0.02	0.81	trivial	unlikely
		POST-+3 h	-47	36	-0.69	0.70	moderate	likely
Total-TIF-1A	HIT+RT vs. RT	PRE-POST	-14	17	-0.37	0.47	small	possibly
		PRE-+1 h	26	52	0.35	0.59	small	possibly
		PRE-+3 h	32	75	0.26	0.52	small	possibly
		POST-+1 h	24	41	0.52	0.78	small	possibly
		POST-+3 h	17	69	0.39	1.34	small	possibly
	MICT+RT vs. RT	PRE-POST	-4	28	-0.09	0.64	trivial	unlikely
		PRE-+1 h	-39	76	-0.32	0.68	small	possibly
		PRE-+3 h	247	431	0.77	0.64	moderate	likely
		POST-+1 h	-40	75	-1.15	2.36	moderate	possibly
		POST-+3 h	49	56	0.90	0.83	moderate	likely
	HIT+RT vs. MICT+RT	PRE-POST	12	31	0.26	0.62	small	possibly
		PRE-+1 h	-43	71	-0.35	0.65	small	possibly
		PRE-+3 h	245	439	0.70	0.60	moderate	likely
		POST-+1 h	-52	60	-1.65	2.38	large	likely
		POST-+3 h	27	73	0.54	1.24	small	possibly

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
p-UBF	HIT+RT vs. RT	PRE-POST	22	58	0.28	0.64	small	possibly
		PRE-+1 h	-30	25	-0.46	0.45	small	possibly
		PRE-+3 h	-56	22	-1.06	0.62	moderate	very likely
		POST-+1 h	-32	23	-0.54	0.46	small	possibly
		POST-+3 h	-49	17	-0.92	0.45	moderate	very likely
	MICT+RT vs. RT	PRE-POST	30	63	0.35	0.62	small	possibly
		PRE-+1 h	-35	29	-0.53	0.53	small	possibly
		PRE-+3 h	-67	17	-1.40	0.62	large	very likely
		POST-+1 h	-37	27	-0.61	0.55	moderate	possibly
		POST-+3 h	-64	12	-1.35	0.42	large	most likely
	HIT+RT vs. MICT+RT	PRE-POST	7	36	0.14	0.69	trivial	possibly
		PRE-+1 h	-21	33	-0.42	0.72	small	possibly
		PRE-+3 h	-47	19	-0.84	0.48	moderate	likely
		POST-+1 h	-7	31	-0.15	0.70	trivial	possibly
		POST-+3 h	-30	16	-0.74	0.48	moderate	likely
Total-UBF	HIT+RT vs. RT	PRE-POST	9	18	0.21	0.41	small	unlikely
		PRE-+1 h	14	24	0.25	0.38	small	unlikely
		PRE-+3 h	2	21	0.03	0.34	trivial	very unlikely
		POST-+1 h	15	26	0.36	0.57	small	possibly
		POST-+3 h	10	29	0.23	0.65	small	possibly

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	MICT+RT vs. RT	PRE-POST	18	15	0.51	0.39	small	possibly
		PRE-+1 h	-2	22	-0.04	0.44	trivial	unlikely
		PRE-+3 h	11	26	0.19	0.41	trivial	unlikely
		POST-+1 h	-1	24	-0.04	0.76	trivial	unlikely
		POST-+3 h	3	26	0.08	0.78	trivial	unlikely
	HIT+RT vs. MICT+RT	PRE-POST	8	19	0.18	0.38	trivial	unlikely
		PRE-+1 h	-22	16	-0.46	0.37	small	possibly
		PRE-+3 h	-7	23	-0.13	0.45	trivial	unlikely
		POST-+1 h	-14	19	-0.35	0.49	small	possibly
		POST-+3 h	-6	23	-0.15	0.54	trivial	unlikely
Total Cyclin D1	HIT+RT vs. RT	PRE-POST	-11	25	-0.11	0.26	trivial	very unlikely
		PRE-+1 h	-21	19	-0.16	0.16	trivial	very unlikely
		PRE-+3 h	75	55	0.36	0.20	small	possibly
		POST-+1 h	-22	19	-0.23	0.23	small	unlikely
		POST-+3 h	15	28	0.14	0.23	trivial	very unlikely
	MICT+RT vs. RT	PRE-POST	-16	15	-0.18	0.18	trivial	very unlikely
		PRE-+1 h	-1	32	-0.01	0.25	trivial	very unlikely
		PRE-+3 h	82	57	0.47	0.24	small	possibly
		POST-+1 h	-2	32	-0.02	0.33	trivial	very unlikely
		POST-+3 h	51	61	0.42	0.40	small	possibly

Protein target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	-7	27	-0.09	0.39	trivial	unlikely
		PRE-+1 h	41	48	0.34	0.33	small	possibly
		PRE-+3 h	77	56	0.50	0.27	small	possibly
		POST-+1 h	25	45	0.31	0.47	small	possibly
		POST-+3 h	31	49	0.36	0.50	small	possibly

mRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
MuRF-1 mRNA	HIT+RT vs. RT	PRE-POST	7	41	0.15	0.81	trivial	possibly
		POST-+3 h	168	176	2.15	1.34	very large	very likely
		PRE-+3 h	187	148	2.29	1.07	very large	most likely
	MICT+RT vs. RT	PRE-POST	35	56	0.57	0.78	small	possibly
		POST-+3 h	7	91	0.13	1.49	trivial	possibly
		PRE-+3 h	44	139	0.70	1.64	moderate	possibly
	HIT+RT vs. MICT+RT	PRE-POST	26	49	0.47	0.77	small	possibly
		POST-+3 h	60	34	1.85	1.56	large	likely
		PRE-+3 h	50	50	1.39	1.78	large	likely
Atrogin-1 mRNA	HIT+RT vs. RT	PRE-POST	0	48	0.01	0.88	trivial	unlikely
		POST-+3 h	89	83	1.22	0.82	large	likely
		PRE-+3 h	90	70	1.22	0.69	large	very likely
	MICT+RT vs. RT	PRE-POST	3	67	0.05	1.13	trivial	possibly
		POST-+3 h	86	89	1.14	0.85	moderate	likely
		PRE-+3 h	91	106	1.19	0.97	moderate	likely
	HIT+RT vs. MICT+RT	PRE-POST	2	60	0.04	1.06	trivial	possibly
		POST-+3 h	-1	42	-0.03	0.80	trivial	unlikely
		PRE-+3 h	1	53	0.02	0.96	trivial	unlikely

mRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
PGC-1α mRNA	HIT+RT vs. RT	PRE-POST	20	34	0.44	0.67	moderate	possibly
		POST-+3 h	635	360	4.80	1.14	extremely large	most likely
		PRE-+3 h	781	413	5.24	1.09	extremely large	most likely
	MICT+RT vs. RT	PRE-POST	-25	59	-0.46	1.18	small	possibly
		POST-+3 h	447	379	2.75	1.05	very large	most likely
		PRE-+3 h	311	425	2.29	1.47	very large	very likely
	HIT+RT vs. MICT+RT	PRE-POST	-37	50	-0.85	1.32	moderate	possibly
		POST-+3 h	-26	50	-0.53	1.14	small	possibly
		PRE-+3 h	-53	47	-1.38	1.60	large	likely
TIF-1A mRNA	HIT+RT vs. RT	PRE-POST	-2	18	-0.06	0.55	trivial	unlikely
		POST-+3 h	-17	26	-0.57	0.93	small	possibly
		PRE-+3 h	-18	27	-0.62	1.01	moderate	possibly
	MICT+RT vs. RT	PRE-POST	-2	17	-0.07	0.53	trivial	unlikely
		POST-+3 h	7	25	0.22	0.71	small	possibly
		PRE-+3 h	5	21	0.15	0.63	trivial	unlikely
	HIT+RT vs. MICT+RT	PRE-POST	0	16	-0.02	0.51	trivial	unlikely
		POST-+3 h	29	45	0.80	1.06	moderate	possibly
		PRE-+3 h	28	42	0.78	1.01	moderate	possibly

mRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
Cyclin D1 mRNA	HIT+RT vs. RT	PRE-POST	33	146	0.28	0.95	small	possibly
		POST-+3 h	0	49	0.00	0.47	trivial	unlikely
		PRE-+3 h	33	168	0.28	1.06	small	possibly
	MICT+RT vs. RT	PRE-POST	-10	107	-0.10	0.94	trivial	possibly
		POST-+3 h	-5	45	-0.05	0.43	trivial	unlikely
		PRE-+3 h	-15	108	-0.15	0.99	trivial	possibly
	HIT+RT vs. MICT+RT	PRE-POST	-32	39	-0.50	0.71	small	possibly
		POST-+3 h	-5	48	-0.07	0.63	trivial	unlikely
		PRE-+3 h	-36	43	-0.57	0.81	small	possibly
Fox-O1 mRNA	HIT+RT vs. RT	PRE-POST	30	246	0.24	1.27	small	possibly
		POST-+3 h	141	73	0.80	0.27	moderate	very likely
		PRE-+3 h	214	560	1.04	1.22	moderate	likely
	MICT+RT vs. RT	PRE-POST	6	36	0.12	0.72	trivial	unlikely
		POST-+3 h	28	74	0.52	1.17	small	possibly
		PRE-+3 h	35	87	0.64	1.30	moderate	possibly
	HIT+RT vs. MICT+RT	PRE-POST	19	155	0.18	1.19	trivial	possibly
		POST-+3 h	47	31	0.54	0.47	small	possibly
		PRE-+3 h	57	82	0.72	1.19	moderate	possibly

mRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
POLR1B mRNA	HIT+RT vs. RT	PRE-POST	37	30	0.87	0.60	moderate	likely
		POST-+3 h	34	51	0.81	1.03	moderate	likely
		PRE-+3 h	83	57	1.69	0.86	large	very likely
	MICT+RT vs. RT	PRE-POST	29	33	0.51	0.51	small	possibly
		POST-+3 h	38	50	0.64	0.72	moderate	possibly
		PRE-+3 h	77	52	1.14	0.58	moderate	very likely
	HIT+RT vs. MICT+RT	PRE-POST	-6	19	-0.15	0.48	trivial	unlikely
		POST-+3 h	3	39	0.06	0.90	trivial	possibly
		PRE-+3 h	-3	31	-0.08	0.76	trivial	unlikely

rRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
Total RNA	HIT+RT vs. RT	PRE-POST	106	67	1.35	0.60	large	most likely
	MICT+RT vs. RT	PRE-POST	69	45	1.05	0.53	moderate	very likely
	HIT+RT vs. MICT+RT	PRE-POST	-14	26	-0.42	0.84	small	possibly
45S pre-rRNA	HIT+RT vs. RT	PRE-POST	58	76	0.71	0.71	moderate	likely
		POST-+3 h	50	97	0.63	0.94	moderate	possibly
		PRE-+3 h	138	169	1.34	1.02	large	likely
	MICT+RT vs. RT	PRE-POST	75	81	0.85	0.68	moderate	likely
		POST-+3 h	27	63	0.36	0.72	small	possibly
		PRE-+3 h	123	133	1.21	0.86	large	likely
	HIT+RT vs. MICT+RT	PRE-POST	11	30	0.18	0.48	trivial	possibly
		POST-+3 h	-16	56	-0.30	1.11	small	possibly
		PRE-+3 h	-6	63	-0.12	1.13	trivial	possibly
5.8S rRNA	HIT+RT vs. RT	PRE-POST	125	109	1.27	0.73	large	very likely
		POST-+3 h	14	80	0.20	1.02	small	possibly
		PRE-+3 h	156	215	1.47	1.20	large	likely
	MICT+RT vs. RT	PRE-POST	120	111	0.99	0.61	moderate	likely
		POST-+3 h	19	80	0.22	0.79	small	possibly
		PRE-+3 h	161	207	1.20	0.91	large	likely

rRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	-2	35	-0.03	0.48	trivial	unlikely
		POST-+3 h	4	77	0.06	0.95	trivial	possibly
		PRE-+3 h	2	93	0.03	1.12	trivial	possibly
5.8S rRNA (span)	HIT+RT vs. RT	PRE-POST	112	116	1.40	0.97	large	very likely
		POST-+3 h	51	82	0.76	0.96	moderate	possibly
		PRE-+3 h	198	161	2.03	0.96	very large	very likely
	MICT+RT vs. RT	PRE-POST	53	86	0.74	0.92	moderate	possibly
		POST-+3 h	27	61	0.41	0.80	small	possibly
		PRE-+3 h	95	98	1.15	0.84	moderate	likely
	HIT+RT vs. MICT+RT	PRE-POST	-28	48	-0.55	1.05	small	possibly
		POST-+3 h	-16	51	-0.29	0.96	small	possibly
		PRE-+3 h	-35	42	-0.72	1.02	moderate	possibly
18S rRNA	HIT+RT vs. RT	PRE-POST	-51	187	-0.45	1.30	small	possibly
		POST-+3 h	39	76	0.21	0.33	small	unlikely
		PRE-+3 h	-32	230	-0.25	1.22	small	possibly
	MICT+RT vs. RT	PRE-POST	-63	145	-0.56	1.17	small	possibly
		POST-+3 h	-1	52	-0.01	0.28	trivial	very unlikely
		PRE-+3 h	-64	124	-0.57	1.09	small	possibly

rRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	-25	48	-0.43	0.90	small	possibly
		POST-+3 h	-29	35	-0.52	0.72	small	possibly
		PRE-+3 h	-47	34	-0.95	0.91	moderate	likely
18S rRNA (span)	HIT+RT vs. RT	PRE-POST	95	156	0.52	0.57	small	possibly
		POST-+3 h	15	102	0.11	0.62	trivial	unlikely
		PRE-+3 h	125	253	0.63	0.75	moderate	possibly
	MICT+RT vs. RT	PRE-POST	59	72	0.32	0.30	small	possibly
		POST-+3 h	26	88	0.16	0.45	trivial	unlikely
		PRE-+3 h	101	137	0.48	0.44	small	possibly
	HIT+RT vs. MICT+RT	PRE-POST	-18	61	-0.13	0.45	trivial	unlikely
		POST-+3 h	9	77	0.06	0.42	trivial	unlikely
		PRE-+3 h	-11	103	-0.07	0.64	trivial	unlikely
28S rRNA	HIT+RT vs. RT	PRE-POST	73	55	1.23	0.71	large	very likely
		POST-+3 h	14	60	0.28	1.13	small	possibly
		PRE-+3 h	97	115	1.52	1.24	large	likely
	MICT+RT vs. RT	PRE-POST	63	55	1.10	0.74	moderate	likely
		POST-+3 h	1	42	0.02	0.92	trivial	unlikely
		PRE-+3 h	65	76	1.12	1.00	moderate	likely

rRNA target	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
	HIT+RT vs. MICT+RT	PRE-POST	-6	21	-0.18	0.69	trivial	possibly
		POST-+3 h	-11	47	-0.36	1.58	small	possibly
		PRE-+3 h	-16	52	-0.54	1.81	small	possibly
28S rRNA (span)	HIT+RT vs. RT	PRE-POST	123	109	0.81	0.48	moderate	likely
		POST-+3 h	24	70	0.22	0.54	small	possibly
		PRE-+3 h	153	136	0.93	0.52	moderate	very likely
	MICT+RT vs. RT	PRE-POST	58	66	0.50	0.45	small	possibly
		POST-+3 h	45	68	0.41	0.50	small	possibly
		PRE-+3 h	128	127	0.91	0.59	moderate	likely
	HIT+RT vs. MICT+RT	PRE-POST	-29	36	-0.65	0.92	moderate	possibly
		POST-+3 h	17	60	0.29	0.93	small	possibly
		PRE-+3 h	-10	58	-0.20	1.15	small	possibly

Measure	Comparison	Change between	Mean difference in change		Standardised effect size (ES)		Effect magnitude	Qualitative likelihood of true effect magnitude being substantial
			% difference	±90% CL	ES (*d*)	±90% CL		
Type I fibre CSA	HIT+RT vs. RT	PRE-POST	-34	22	-1.03	0.80	moderate	likely
	MICT+RT vs. RT	PRE-POST	-15	54	-0.39	1.45	small	possibly
	HIT+RT vs. MICT+RT	PRE-POST	29	86	0.63	1.53	moderate	possibly
Type II fibre CSA	HIT+RT vs. RT	PRE-POST	-15	23	-0.43	0.69	small	possibly
	MICT+RT vs. RT	PRE-POST	-2	32	-0.07	0.92	trivial	possibly
	HIT+RT vs. MICT+RT	PRE-POST	16	39	0.42	0.97	small	possibly