



Whole-body and skeletal muscle responses to divergent modes of exercise training and detraining in middle-aged men

Submitted by

Marcus Callahan, B.App.Sci. (Ex&SportSc), B.Ex.SSc (Hons)

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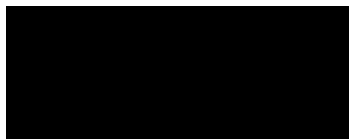
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The extent to which other persons contributed to work arising from this thesis is specified in **Appendix C**.



Marcus J. Callahan

Date: 01/02/2021

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Callahan, M.J., Whitfield, J., Parr, E.B., Hawley, J.A., Camera, D.M. Gene and protein expression responses implicated in exercise training adaptations following short-term exercise training and detraining in middle-aged men.

CONFERENCE PROCEEDINGS

Marcus J. Callahan, Evelyn B. Parr, Miguel S. Conceição, John A. Hawley and Donny M. Camera. Short-term training and detraining responses following divergent exercise modalities in middle-aged men. European College of Sport Sciences, July 2019, Prague, Czech Republic.

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LIST OF ABBREVIATIONS

1RM	One-repetition maximum
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
ActRIIB	Activin receptor type-2B
ADP	Air displacement plethysmography
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
Atrogin-1	Muscle atrophy F-box
AUC	Area under the curve
BM	Body mass
BMI	Body mass index
CaMKII	Calcium/calmodulin-dependent protein kinase II
CHO	Carbohydrate
CPT1b	Carnitine palmitoyltransferase
CSA	Cross-sectional area
CT	Computed topography
CT	Combined training
D ₂ O	Deuterium oxide
DEXA	Dual energy x-ray absorptiometry
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EMG	Electromyography
ENT	Endurance exercise training
FFM	Fat-free mass
FOXO3a	Forkhead box O3
h	Hour
HIIT	High-intensity interval training
HOMA2-IR	Homeostatic model assessment of insulin resistance
HR _{peak}	Peak heart rate
IRS	Insulin-like growth factor
LM	Lean mass
MAP	Maximal aerobic power
MEF	Myocyte enhancer factor
min	Minute

MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MSTN	Myostatin
MT	Muscle thickness
mtDNA	Mitochondrial DNA
mTOR	Mechanistic target of rapamycin
mTORC1	Mechanistic target of rapamycin complex 1
MuRF-1	Muscle-ring Finger 1
MYOD1	Myogenic differentiation 1
N m	Newton metres
NR4A3	Nuclear Receptor Subfamily 4 Group A Member 3
NRF	Nuclear respiratory factor
OGTT	Oral glucose tolerance test
p38 MAPK	p38 mitogen-activated protein kinase
p53	Tumour suppressor protein p53
p70S6K1	Ribosomal protein of 70-kDa S6 kinase 1
PGC-1 α	Proliferator activated receptor γ coactivator-1 α
PI3K	Phosphatidylinositol 3-kinase
PIM	Protein import machinery
PPAR	Peroxisome proliferator-activated receptor
PPO	Peak power output
QF	<i>Quadriceps femoris</i>
REE	Resting energy expenditure
RET	Resistance exercise training
RMR	Resting metabolic rate
RNA	Ribonucleic acid
rpS6	Ribosomal protein S6
Ser	Serine
SC	Satellite cell
SD	Standard deviation
SIT	Sprint interval training
Thr	Threonine

Tfam	Mitochondrial transcription factor
UPS	Ubiquitin–proteasome system
UWW	Underwater weighing
VEGF	Vascular endothelial growth factor
VI	<i>Vastus intermedius</i>
VL	<i>Vastus lateralis</i>
VO _{2max}	Maximal oxygen uptake
VO _{2peak}	Peak oxygen uptake
y	Years

ABSTRACT

While short-term (~six weeks) HIIT induces rapid increases in skeletal muscle oxidative capacity, the anabolic potential of HIIT for promoting concurrent gains in skeletal muscle mass has received less scientific inquiry. The experiments undertaken for this thesis investigated skeletal muscle adaptive responses following HIIT, resistance exercise training (RET) and endurance exercise training (ENT), and after a subsequent period of detraining, in sedentary, middle-aged men. Thirty-five sedentary, males (39 ± 3 y) performed six weeks of either ENT (n=12), HIIT (n=12) or RET (n=11) followed by 2.5 weeks of detraining. Skeletal muscle gene and protein expression, muscle fiber characteristics, body composition, muscle thickness, muscle strength, aerobic capacity, resting metabolic rate and glucose control were assessed at baseline, and after exercise training and detraining.

Lean mass increased after RET and HIIT ($+3.2 \pm 1.6\%$ and $+1.6 \pm 2.1\%$, $P < 0.05$). Muscle strength (sum of leg press, leg extension and bench press 1RMs) increased after all exercise training interventions (RET: $+25 \pm 5\%$; HIIT: $+10 \pm 5\%$; ENT: $+7 \pm 7\%$, $P < 0.05$). Aerobic capacity increased only after HIIT and ENT ($+14 \pm 7\%$ and $+11 \pm 11\%$, $P < 0.05$). Type I and II muscle fiber size increased for all groups after exercise training and remained elevated after detraining (main effect of time, $P < 0.05$). Following detraining, the gains in lean mass and muscle strength were maintained in RET and HIIT groups, but maximal aerobic capacity declined below post-exercise training levels in HIIT and ENT ($P < 0.05$). Androgen receptor, Akt and mTOR total protein increased after exercise training in all groups. Vitamin D receptor and apelin receptor mRNA increased after exercise training in all groups (main effect of time, $P < 0.05$).

Six weeks of HIIT resulted in the most pronounced skeletal muscle adaptation prior to detraining in middle-aged men. While only HIIT and RET resulted in increases in lean mass and muscle thickness, gene and protein expression of markers implicated in muscle growth

responses were largely similar across all exercise training modes. Short-term detraining did not negatively impact gains in muscle size, mass or strength irrespective of exercise modality.

CHAPTER 1

Introduction, Overview & Literature review

Section 1.4 of this chapter has been adapted from the following published review:

Callahan, M.J., Parr, E.B., Hawley, J.A., Camera, D.M. Can high-intensity interval training promote skeletal muscle anabolism? *Sports Medicine* (2021). doi: 10.1007/s40279-020-01397-3.

1.1 INTRODUCTION

Human skeletal muscle comprises ~40% of body mass and plays fundamental roles in locomotion, thermoregulation and metabolic health (Cartee *et al.*, 2016; Zierath & Hawley, 2004). The age-related loss of skeletal muscle mass and function, a clinical condition termed sarcopenia, commences during middle adulthood (~40 y) and is underpinned by decreases in the number and size of predominantly fast-twitch muscle fibers (Lexell *et al.*, 1988; Wilkinson *et al.*, 2018). The progression of sarcopenia is influenced by several environmental factors (e.g., level of habitual physical activity, nutrition) that act independently, or synergistically to mediate skeletal muscle protein homeostasis throughout adulthood. Indeed, peak skeletal muscle mass attained during middle-age is related to skeletal muscle mass and strength in older adults (Sayer *et al.*, 2008). The consequences of sarcopenia, such as loss of independence and increased morbidity/mortality, are most evident in older age (>60 y) (Arango-Lopera *et al.*, 2013; Wang *et al.*, 2020). Thus, maintenance of lean (i.e., skeletal muscle) mass with advancing age is a vital component of healthy aging (Li *et al.*, 2018; Roubenoff, 2003). Indeed, lean mass is an important predictor of mobility as well as glycaemic homeostasis (Lee *et al.*, 2017a; Reid *et al.*, 2008). However, as age-related skeletal muscle loss begins to manifest during middle adulthood, it is important to understand how middle-aged adults (defined herein as between the ages of 40-60 y) respond to stimuli known to promote accrual and loss of skeletal muscle.

Resistance exercise training (RET; e.g., machine-based or free weights), characterised by repeated strength/power-based movements against external loads, is the primary exercise modality for increasing skeletal muscle mass in humans. On the other hand, endurance exercise training (ENT; e.g., running, cycling), defined by continuous or interval-style exercise at submaximal intensities, primarily increases skeletal muscle oxidative capacity and whole-body cardiorespiratory fitness (Coffey & Hawley, 2017). More recently, ENT has been shown to increase lean mass (Jonvik *et al.*, 2019; Knuiman *et al.*, 2019), suggesting exercise modalities in addition to traditional strength-based exercise training can increase and/or maintain skeletal

muscle mass. However, despite the well-established physical and psychological health benefits associated with resistance and endurance exercise, respectively, a ‘lack of time’, due to competing priorities (e.g., family, work and social commitments), remains the most common barrier to participation in regular exercise training programmes (Dishman *et al.*, 1985; Franco *et al.*, 2015; Trost *et al.*, 2002). Thus, exercise modalities that can be completed in a time-efficient manner (e.g., <30 min per session) may assist ‘time-poor’ individuals to meet general population exercise guidelines.

One form of ‘time-friendly’ exercise training is high-intensity interval training (HIIT) which comprises short periods of intense (80-100% peak heart rate [HR_{peak}]) aerobic exercise (e.g., cycling, running, rowing) interspersed by low intensity exercise (i.e., active) recovery or rest (Weston *et al.*, 2014). HIIT induces a similar skeletal muscle phenotype to that observed after ENT, namely an enhanced cardiorespiratory fitness and maximal power output (MacInnis & Gibala, 2017). Short-term HIIT (i.e., ~2-6 weeks) induces rapid increases in mitochondrial biogenesis, maximal oxygen uptake (VO_{2max}) (Baekkerud *et al.*, 2016; Gillen *et al.*, 2013; Perry *et al.*, 2010) and short-term cycling performance (i.e., 50 kJ and 750 kJ time trials) (Little *et al.*, 2010b). While HIIT has several similarities to RET (e.g., repeated work-rest cycles placing skeletal muscle under high intermittent stress), little is known about skeletal muscle growth promoting (i.e., anabolic) responses with short-term (i.e., 4-8 weeks) HIIT. Specifically, it is not known whether short-term HIIT induces skeletal muscle fiber hypertrophy (i.e., increases in the cross-sectional area (CSA) of individual fibers) in middle-aged adults. Additionally, comparisons of skeletal muscle fiber size responses following short-term HIIT, RET and ENT have not been examined in the same study. In this regard, the majority of studies quantifying skeletal muscle fiber CSA responses with exercise training have been of longer duration (~3-6 months).

The investigation of short-term (4-8 weeks) adaptative responses with divergent exercise training modes represents an important, but understudied area of the skeletal muscle

literature. For instance, many individuals undergo surgery at short notice (i.e., 1-2 months), and do not have the time to undertake prolonged (i.e., ≥ 3 months) exercise training regimens before they are hospitalised (Topal *et al.*, 2019). Moreover, many individuals who exercise train recreationally only engage in short-term programmes before either switching to another program or temporarily/permanently ceasing to exercise at all (Wenger & Bell, 1986). As such, it is important to determine the short-term, mode-specific effects of exercise training on multiple components of physical fitness to help form evidence-based guidelines for recommendations concerning cases of short-term pre-operative exercise training programming.

Table 1.1 Classification of exercise training programme durations to distinguish short-term from prolonged exercise training in this thesis.

	Short-term	Prolonged
Duration (weeks)	4-8	≥ 12

Another knowledge gap regarding skeletal muscle adaptation responses is the effects of exercise training cessation (i.e., detraining) following different exercise training modalities. Detraining is defined as a partial or complete loss of exercise training-induced adaptations due to a reduction or cessation in exercise frequency, intensity or duration (Mujika & Padilla, 2000). In the context of exercise training and skeletal muscle anabolism/catabolism (i.e., changes in mass and/or size), prolonged periods of detraining ($\geq$ four weeks) (Mujika & Padilla, 2000) have received extensive investigation (Blocquiaux *et al.*, 2020; Brandner *et al.*, 2019; Kubo *et al.*, 2010; Lo *et al.*, 2011; Psilander *et al.*, 2019; Spence *et al.*, 2011; Taaffe & Marcus, 1997) compared to short-term (~2-4 weeks) periods of rest or detraining (Hwang *et al.*, 2017; Kadi *et al.*, 2004; McMahon *et al.*, 2019). Furthermore, the effects of brief periods of exercise training cessation (~2-3 weeks) on skeletal anabolism have, for the most part, been reported in predominantly well-trained individuals (Mujika & Padilla, 2001). Like athletes, adults may also

face short unavoidable periods of exercise training cessation (e.g., illness, injury, vacation). However, skeletal muscle fiber size responses following short-term detraining that proceeds single-mode exercise (i.e., ENT, HIIT and RET) have not been investigated in middle-aged adults. Additionally, further scrutiny of the molecular regulation of skeletal muscle mass in humans is required to better understand the mechanisms that modulate muscle hypertrophy and atrophy in response to short-term exercise training and detraining.

This literature review commences with a discussion of molecular and skeletal muscle remodelling processes in response to short-term RET and aerobic-based (i.e., ENT and HIIT) exercise training modes, along with the role of dietary interventions (i.e., protein supplementation) to augment skeletal muscle adaptations to exercise. Subsequently, investigations that have determined the capacity for short-term HIIT and the interaction with protein ingestion to induce skeletal muscle anabolism from cellular, fiber and whole muscle levels will be discussed. Finally, a review of the effects of short-term periods of detraining on anabolic human skeletal muscle adaptations following either RET, ENT or HIIT will be examined.

1.2 RESISTANCE EXERCISE TRAINING & SKELETAL MUSCLE ANABOLISM

1.2.1 Molecular Responses Implicated in Skeletal Muscle Anabolism and Catabolism after Resistance Exercise Training

Resistance exercise induces mechanical stress to trigger skeletal muscle cell sensors that transduce signals to targets (e.g., proteins, transcription factors) regulating the synthesis and breakdown of predominantly myofibrillar proteins (i.e., turnover) (Egan & Zierath, 2013). Repeated loading of these molecular programs facilitates positive net protein balance, where rates of muscle protein synthesis (MPS) exceed rates of protein breakdown. In turn, prolonged RET leads to increases in skeletal muscle fiber size and muscle mass which is related to enhanced force generating capacity against an external load (i.e., strength) (McGlory *et al.*,

2017a). Accordingly, RET is viewed as the ‘gold standard’ across all age-groups for increasing, or regaining, skeletal muscle (Phillips, 2014).

Increases in MPS induced by resistance exercise are largely mediated by increases in the phosphorylation of mechanistic target of rapamycin complex 1 (mTORC1) (Goodman *et al.*, 2011). mTORC1 in turn stimulates the activity of protein kinases such as the ribosomal protein of 70-kDa S6 kinase 1 (p70S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) that are both involved in translation initiation (Shah *et al.*, 2000) (**Figure 1.1**). Specifically, p70S6K1 regulates the ribosomal biogenesis transcriptional program (Chauvin *et al.*, 2014) and 4E-BP1 promotes ribosomal binding to transcribed mRNA necessary for protein translation (Gingras *et al.*, 1999). Repeated activation of these kinases through RET improves the translational capacity (ribosome abundance) and translational efficiency of skeletal muscle cells to promote increased synthesis of myofibrillar proteins. Numerous studies have reported increases within the mTORC1 signalling cascade following acute (i.e., single bout) resistance exercise (Areta *et al.*, 2013; Camera *et al.*, 2010; Dreyer *et al.*, 2006), as well as short-term (Kazior *et al.*, 2016; Leger *et al.*, 2006) and prolonged (Terzis *et al.*, 2008) RET in human skeletal muscle. Previous work has also associated increases in p70S6K1 phosphorylation with acute rates of MPS (Kumar *et al.*, 2009; West *et al.*, 2009) and muscle hypertrophy (Mayhew *et al.*, 2009; Terzis *et al.*, 2008) following RET, although such relationships are not always observed (Mitchell *et al.*, 2012).

The majority of studies of resistance exercise-induced skeletal muscle growth have focused on the Akt (protein kinase B)-mTORC1-p70S6K1 axis due to its putative role in elevating post-exercise rates of skeletal muscle protein synthesis (Laplane & Sabatini, 2012). However, the expression of other genes, proteins and circulating factors (e.g., hormones, myokines) implicated in skeletal muscle anabolism have also been examined following RET (Adams & Bamman, 2012). Binding of growth factors such as testosterone and other androgens to their receptor in skeletal muscle cells initiates their translocation to the nucleus to activate

genes regulating muscle anabolism (Jiang *et al.*, 2009; Wilkenfeld *et al.*, 2018). Previous work from Sato *et al.* (2014) in older (>60 y) men showed skeletal muscle expression of steroidogenesis-related enzymes and androgen protein expression can mediate prolonged RET-induced muscle hypertrophy via autocrine signalling. Six weeks of RET in older adults supplementing with, but not without, testosterone increased androgen receptor mRNA (Gharahdaghi *et al.*, 2019). In contrast, androgen receptor protein content remained unchanged after six weeks of RET in young well-trained men (Haun *et al.*, 2019a). Moreover, increased intramuscular androgen receptor content has been associated with prolonged RET-induced muscle fiber hypertrophy (Ahtiainen *et al.*, 2011; Mitchell *et al.*, 2013; Morton *et al.*, 2018) although not always (Haun *et al.*, 2018; Mobley *et al.*, 2018). In any case, the intramuscular androgen receptor appears to be a key molecular transducer underpinning exercise training-induced muscle hypertrophy (Clarke *et al.*, 2019; Wu *et al.*, 2017; Yin *et al.*, 2020).

Another protein putatively involved in RET-induced increases in skeletal muscle anabolism is the Vitamin D receptor. Studies in skeletal muscle cells show vitamin D supplementation increases expression of muscle regulatory factors myogenic differentiation 1 (MYOD1) and myogenin (Braga *et al.*, 2017; Irazoqui *et al.*, 2014) and stimulates protein synthesis via Akt/mTOR mediated pathways (Salles *et al.*, 2013). Indeed, a meta-analysis of 30 randomised controlled trials reported that Vitamin D supplementation induced a significant positive effect on muscle strength in older adults (Beaudart *et al.*, 2014). Moreover, greater Vitamin D receptor mRNA expression was associated with a greater increase in lean mass following 20 weeks of RET in human skeletal muscle of adults (aged 18-75 y) (Bass *et al.*, 2020).

More recently, circulating levels of the ‘exerkine’ apelin was demonstrated to be positively associated with physical function in older adults as well stimulating protein synthesis in young and aged muscle cells and target muscle stem cells to enhance muscle regeneration (Vinel *et al.*, 2018). Following 10 weeks of RET in recreationally active men (18-30 y), Stokes

et al. (2020) reported increased apelin receptor mRNA which correlated with subsequent muscle growth. Thus, several novel mRNA and cell signalling proteins have been implicated in the molecular mechanisms mediating RET-induced skeletal muscle growth in addition to the more ‘established’ regulators. However, to date, few studies have measured androgen receptor (Gharahdaghi *et al.*, 2019; Haun *et al.*, 2019a), vitamin D receptor or apelin receptor mRNA or protein following short-term RET in human skeletal muscle and none in middle-aged adults.

RET also increases rates of muscle protein breakdown (MPB) in the post-absorptive state such that, even though MPS is stimulated to a greater extent, net protein balance remains negative (Phillips *et al.*, 2002). Given the inherent technical difficulties associated with measuring rates of MPB in human skeletal muscle (Atherton *et al.*, 2016), most investigations have focussed on measuring expression of proteolytic genes and proteins post-RET as surrogate measures of MPB. In this regard, degradation of skeletal muscle proteins is under the regulation of four major proteolytic mechanisms: 1) the ubiquitin–proteasome system (UPS): main regulatory mechanism of protein degradation in skeletal muscle that, through the action of ubiquitin-ligase enzymes (E3), determine the selectivity and specificity of proteins for degradation; 2) calcium-dependent calpains that mediate the regulatory cleavage of specific substrates (Bartoli & Richard, 2005); 3) lysosomal proteolytic system, in which cathepsins are the major lysosomal proteases (Murton *et al.*, 2008); and 4) caspases, another form of specific protease (Du *et al.*, 2004). The ‘atrogenes’ muscle-ring Finger 1 (MuRF-1) and muscle atrophy F-box (atrogin-1) are two types of E3s that have received considerable scientific enquiry following exercise-induced contraction as they are known to target contractile and structural muscle proteins (Foletta *et al.*, 2011). MuRF-1 mRNA is consistently elevated ~1-4 h after a single bout of resistance exercise in either untrained (Raue *et al.*, 2007; Takegaki *et al.*, 2020), recreationally active (Borgenvik *et al.*, 2012; Reitelseder *et al.*, 2014) or well-trained adults (Louis *et al.*, 2007), as is MuRF-1 total protein within the same time-course (Borgenvik *et al.*,

2012). In response to eight weeks of RET, Leger *et al.* (2006) observed increased MuRF-1 mRNA in recreationally active young men. In contrast, no change in MuRF-1 mRNA or total protein content was observed following seven (Kazior *et al.*, 2016) or 10 weeks (Stefanetti *et al.*, 2015) of RET in untrained young men. Given the similarities in post-exercise biopsy sampling time points (~48-72 h) differences in baseline training status of participants (recreationally active vs untrained) from these studies (Kazior *et al.*, 2016; Leger *et al.*, 2006; Stefanetti *et al.*, 2015) may, in part, explain the discrepant results.

Changes in atrogin-1 mRNA expression following a single bout of resistance exercise are equivocal with previous work reporting an increase (Louis *et al.*, 2007; Raue *et al.*, 2007), decrease (Reitelseder *et al.*, 2014) or no change (Mascher *et al.*, 2008). Additionally, no change in atrogin-1 total protein was observed after a single session of resistance exercise in recreationally active young adults (Borgenvik *et al.*, 2012). Following longer training durations (8-21 weeks) of RET, increases (Leger *et al.*, 2006; Stefanetti *et al.*, 2015), decreases (Kazior *et al.*, 2016) or no change (Hulmi *et al.*, 2009; Kazior *et al.*, 2016; Stefanetti *et al.*, 2015) in both atrogin-1 mRNA and total protein have been reported. Taken together, both acute and prolonged periods of RET show equivocal results regarding the gene and protein expression of MuRF-1 and atrogin-1 in young adults. Whether MuRF-1 and atrogin-1 expression is altered following RET in middle-aged adults is unknown.

While most of the focus into RET-induced adaptations has centred on muscle growth and loss, prolonged RET has also been shown to increase maximal oxygen uptake and levels of markers (e.g., citrate synthase, PGC-1 α) indicative of a more oxidative skeletal muscle phenotype (Parry *et al.*, 2020). Yet, few investigations have assessed metabolic skeletal muscle adaptations following short-term RET in middle-aged adults.

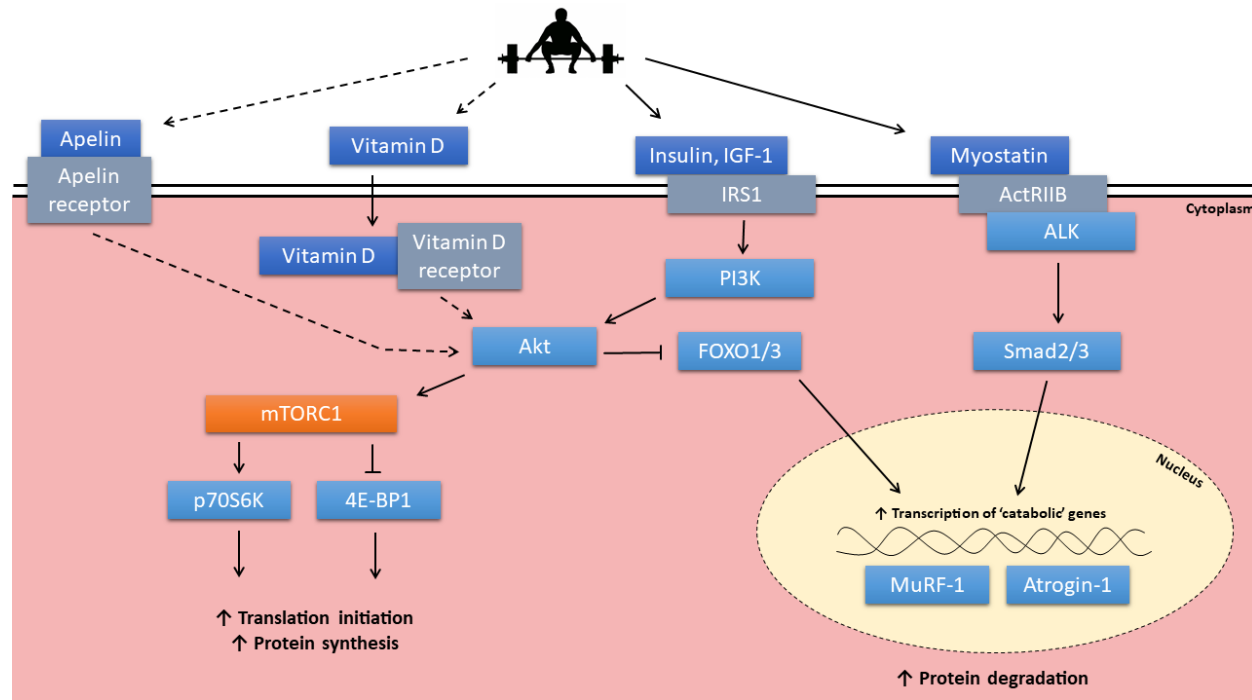


Figure 1.1 Schematic of signalling events induced by resistance exercise in human skeletal muscle.

4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; ActRIIB, activin receptor type-2B; ALK, activin receptor-like kinases; Akt, protein kinase B; atrogin-1, muscle atrophy F-box; IGF, insulin-like growth factor; IRS, insulin receptor substrate; mTORC1, mammalian target of rapamycin complex 1; MuRF-1, muscle-ring Finger 1; p70S6K, ribosomal protein S6 kinase beta-1; PI3K, phosphatidylinositol 3-kinase; VDR, vitamin D receptor. Dotted lines indicate limited research in human skeletal muscle in response to resistance exercise.

1.2.2 Effects of Resistance Exercise Training on Skeletal Muscle Fiber Cross-Sectional Area

Prolonged RET induces skeletal muscle fiber hypertrophy in young (Bellamy *et al.*, 2014; Mackey *et al.*, 2011; Petrella *et al.*, 2006; Reidy *et al.*, 2017; Snijders *et al.*, 2016) and older adults (Frontera *et al.*, 1988; Kadi *et al.*, 2004; Leenders *et al.*, 2013; Mackey *et al.*, 2007; Petrella *et al.*, 2006; Verdijk *et al.*, 2009). In contrast, other studies report no change in skeletal muscle fiber size following prolonged RET in older adults (Blocquiaux *et al.*, 2020; Karlsen *et al.*, 2019; Mero *et al.*, 2013). Regardless, the vast majority of investigations that have determined RET-induced skeletal muscle fiber hypertrophy have been following prolonged training durations (~3-6 months) (Grgic *et al.*, 2019; Peterson *et al.*, 2011; Straight *et al.*, 2020). While such long-term investigations allow for greater potential to observe robust muscle fiber hypertrophy, shorter periods of RET require further inquiry to determine whether lesser periods (e.g., several weeks rather than months) are adequate to induce skeletal muscle fiber hypertrophy.

It has been suggested that skeletal muscle fiber hypertrophy is detectable after as little as six weeks of RET (McGlory *et al.*, 2017a). Earlier ‘increases’ in whole muscle CSA have been largely attributed to artefact such as excess swelling (oedema), particularly in untrained individuals (Damas *et al.*, 2018) (**Figure 1.2**). Several studies in young adults following 4-6 weeks of RET show no change in skeletal muscle fiber hypertrophy (Goreham *et al.*, 1999; Kadi *et al.*, 2004; Lüthi *et al.*, 1986; Sieljacks *et al.*, 2019; Snijders *et al.*, 2016). In contrast, skeletal muscle fiber hypertrophy in young adults has been reported after 6-8 weeks of RET (Bell *et al.*, 2000; de Souza *et al.*, 2014; Goreham *et al.*, 1999; Stasinaki *et al.*, 2015) (**Table 1.2**) suggesting a minimum of six weeks is required to detect skeletal muscle fiber hypertrophy following RET. Indeed, Snijders *et al.* (2016) observed no change in muscle fiber size after four weeks of RET although muscle hypertrophy was detected at week eight (type II only) and 12 (type I and II) of the programme in young men. Compared to prolonged RET interventions in

young adults, limited research exists examining the capacity for short-term RET to induce muscle fiber hypertrophy in middle-aged adults.

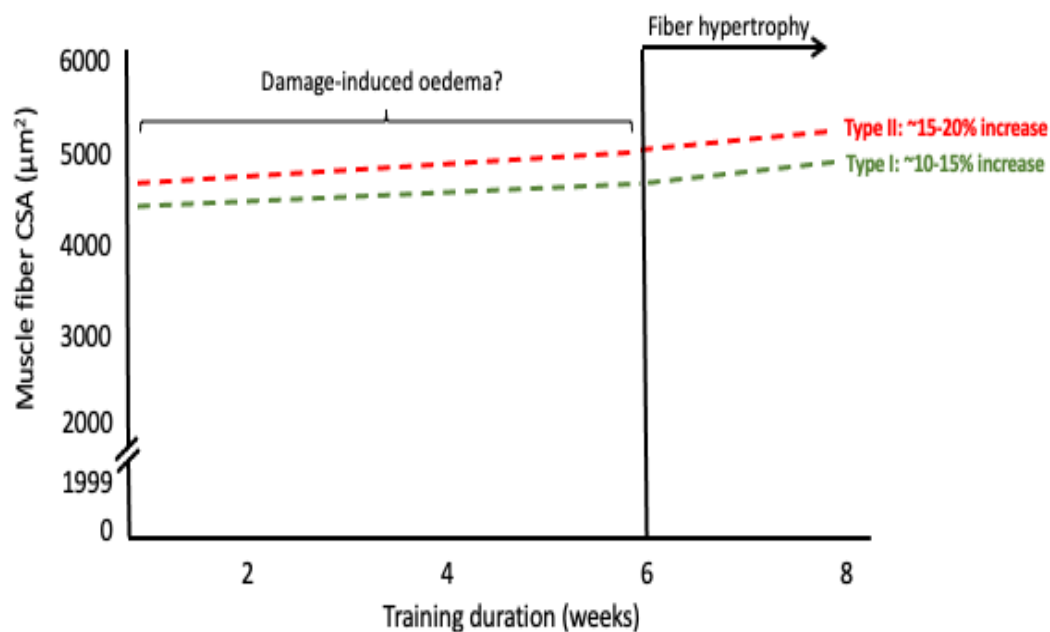


Figure 1.2 Hypothetical model of short-term RET-induced muscle fiber hypertrophy.

Table 1.2 Studies assessing muscle fiber size/morphology after short-term RET.

Study	Total N in RET group (male/female)	Age (y), training status	Intervention duration (weeks)	RET frequency (days·week ⁻¹)	Intensity of training	Nutritional intervention	Key muscle measurements	Main findings
Bell <i>et al.</i> (2000)	11 (7/4)	22 ± 3, recreationally active	12 Measurements also taken at week 6	3	72-84% 1RM	NA	VL muscle fiber CSA	Muscle fiber CSA at week 6: type I ↑ (~15%), type II ↑ (~14%)
Bjørnsen <i>et al.</i> (2019)	8	25 ± 6, resistance-trained	~6	NA	74-76% 1RM	NA	VL muscle fiber CSA, VL muscle thickness	Muscle fiber CSA: type I ↔, type II ↔ VL muscle thickness: : ↔
de Souza <i>et al.</i> (2014)	11	25 ± 6, untrained	8	2	6-12 RM	NA	VL muscle fiber CSA	Muscle fiber CSA: type I ↑ (~17%), type IIa ↑ (~18%), type IIx ↔

Gharahdaghi <i>et al.</i> (2019)	9 (9/0)	70 ± 1, untrained	6	3	80% 1RM	NA	VL muscle fiber CSA, VL muscle thickness	Muscle fiber CSA: type I ↑ (~17%), type IIA ↑ (~17%), type IIX ↔ VL muscle thickness: : ↑
Goreham <i>et al.</i> (1999)	7	20 ± 1, untrained	12 Measurements also taken at week 4 and 7	3	6-8 RM	NA	VL muscle fiber CSA	Mean muscle fiber CSA at week 4: ↔ Mean muscle fiber CSA at week 7: ↑ (5%)
Kadi <i>et al.</i> (2004)	15	24 ± 1, untrained	12 Measurements also taken at week 4	3	6-12 RM	NA	VL muscle fiber CSA	Muscle fiber CSA at week 4: ↔

Kazior <i>et al.</i> (2016)	7 (7/0)	28 ± 4, untrained	7	~3	70-85% 1RM	NA	VL muscle fiber CSA	Muscle fiber CSA: type I ↔, type IIA ↑ (~7%), type IIX ↑ (10%)
Lundberg <i>et al.</i> (2013)	10	25 ± 4, recreationally active	5	3	‘Maximal effort’	NA	VL muscle fiber CSA, QF CSA by MRI	Muscle fiber CSA: type I ↔, type II ↔ QF CSA: ↑
Lundberg <i>et al.</i> (2020)	10	25 ± 4, recreationally active	5	3	NA	NA	VL muscle fiber CSA	Muscle fiber CSA: type I ↔, type II ↑ (~16%)
Lüthi <i>et al.</i> (1986)	8	~18, NA	6	3	NA	NA	VL muscle fiber CSA, midhigh (VL and VI) CSA by CT	Muscle fiber CSA: ↔, midhigh CSA: ↑
Snijders <i>et al.</i> (2016)	22	23 ± 1, recreationally active	12 Measurements also taken at week 4 and 8	3	70-80% 1RM	Post-exercise meal (37 g CHO, 10 g	VL muscle fiber CSA	Muscle fiber CSA at week 4: type I ↔, type II ↔

						protein, 9 g fat) Pre-sleep beverage (15 g CHO, 13.8 g casein, 0.1 g fat)		Muscle fiber CSA at week 8: type I ↔, type II ↑ (~25%)
Sieljacks <i>et al.</i> (2019)	12 (12/0)	~24, untrained	6	3	70-80% 1RM	NA	VL muscle fiber CSA	Muscle fiber CSA: type I ↔, type II ↔
Stasinaki <i>et al.</i> (2015)	9	22 ± 2, untrained	6	3	6 RM	NA	VL muscle fiber CSA, VL and gastrocnemius muscle thickness	Muscle fiber CSA: type I ↑ (~7%), type IIa ↑ (~17%), type IIx ↑ (~11%) VL and gastrocnemius

								muscle thickness: ↑
Staron <i>et al.</i> (1994)	12 (7/5)	21 ± 2, recreationally active	9 Measurements also taken at week 3, 5 and 7	2	6-12 RM	NA	VL muscle fiber CSA	Type I, type IIa and IIb muscle fiber CSA: ↔ at any week

CHO, carbohydrate; CT, computed topography; CSA, cross-sectional area; QF, *quadriceps femoris*; RET, resistance exercise training; VI, *vastus intermedius*; VL, *vastus lateralis*; ↑, significant increase after RET; ↔, unchanged after RET; ↓, significant decrease after RET.

1.2.3 Effects of Resistance Exercise Training on Body Composition and Whole-Muscle Morphology

When measuring RET-induced hypertrophy at the whole-muscle level, several different assessment methods have been commonly utilised in the literature (Haun *et al.*, 2019b). These include dual energy x-ray absorptiometry (DEXA; measurements of lean/fat-free mass), computed topography and magnetic resonance imaging (MRI; measurements of whole-muscle CSA and muscle volume), and ultrasound (measurements of muscle volume and thickness) (Figure 1.3).

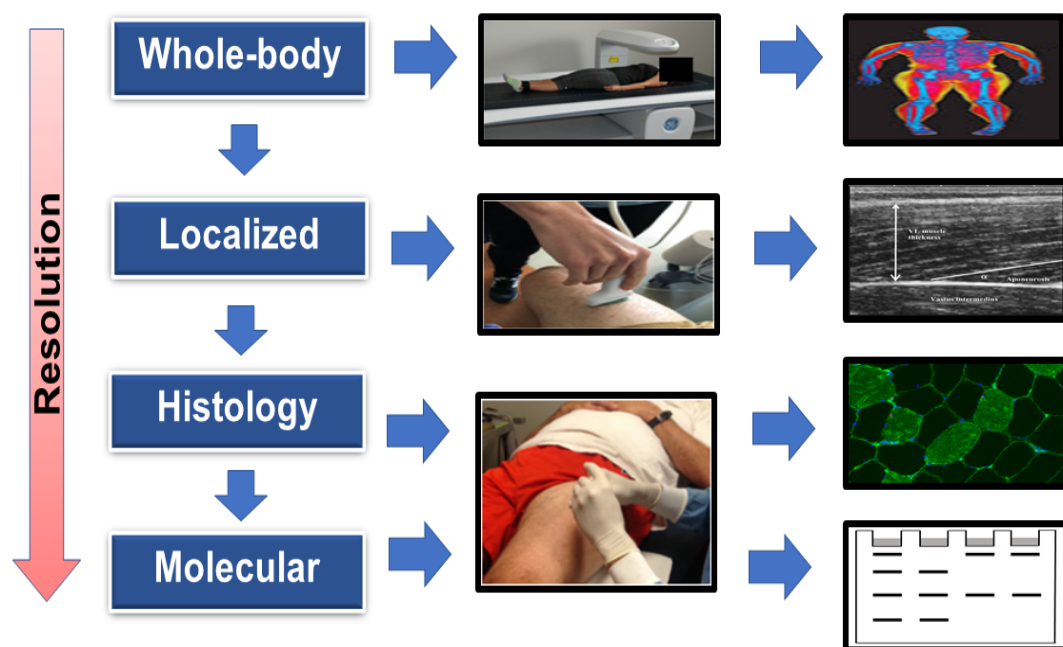


Figure 1.3 Techniques used to assess exercise training-induced changes in skeletal muscle growth from whole-body to molecular (i.e., cell signalling proteins regulating translation initiation including, but not limited to, mTORC1, p70S6K and 4E-BP1) levels.

Numerous studies have investigated changes in whole-body and regional lean/fat-free mass in younger and older adults after short-term RET (**Table 1.3**). Compared to young and older adults, less is known about the effects of short-term RET on lean/fat-free mass responses in middle-aged adults. As expected, heterogenous responses regarding the magnitude of

changes in lean/fat-free mass have been reported. Some of the larger increases in total lean/fat-free mass following short-term RET are in the range of 2-3 kg (Paoli *et al.*, 2017; Rozenek *et al.*, 2002; Saremi *et al.*, 2010), although most studies have measured more modest increases of ~0.5-1.5 kg (Alcaraz *et al.*, 2011; Antonio *et al.*, 2015; Candow & Burke, 2007; Escalante *et al.*, 2016; Gobbo *et al.*, 2013). Several studies report no change in total lean/fat-free mass after short-term RET (Antonio *et al.*, 2014; Ara *et al.*, 2006; Avelar *et al.*, 2019; Coburn *et al.*, 2006; Fyfe *et al.*, 2016; Scanlon *et al.*, 2014; Spillane & Willoughby, 2016; Tinsley *et al.*, 2017; Weisgarber *et al.*, 2012). In two studies (Avelar *et al.*, 2019; Fyfe *et al.*, 2016), increases in lower body lean mass have been reported in the absence of a change in total lean mass. The equivocal results observed following short-term RET may be explained by differences in the duration of the intervention, RET protocol (e.g., lower- vs whole-body), techniques used to assess lean/fat-free mass (e.g., DEXA, air displacement plethysmography, underwater weighing) and the initial training status of participants (e.g., untrained vs resistance trained).

Short-term RET has been shown to increase thigh muscle thickness in young (Avelar *et al.*, 2019; Brook *et al.*, 2015; Weisgarber *et al.*, 2012) and older adults (Gharahdaghi *et al.*, 2019; Scanlon *et al.*, 2014). Similarly, increases in *vastus lateralis* CSA (ultrasound) (Seynnes *et al.*, 2007), *quadriceps femoris* CSA (MRI) (Coburn *et al.*, 2006; Lundberg *et al.*, 2013; Seynnes *et al.*, 2007), *triceps brachii* and *pectoralis major* CSA (MRI) (Ogasawara *et al.*, 2011) have been observed following 5-8 weeks of RET in young adults. Taken collectively, the results from these studies show that changes to lower and upper body muscle morphology, indicative of increased muscle size, are attainable with short-term RET. While most studies have reported such changes in young adults, it is not known whether similar muscle morphology changes occur in middle-aged adults following short-term RET.

Table 1.3 Studies assessing lean/fat-free mass after short-term RET.

Study	Total N in RET group (male/female)	Age (years), training status	Intervention duration (weeks)	RET frequency (days·week ⁻¹)	Intensity of training	Nutrition intervention	Lean/fat-free mass measurement	Main findings after short-term RET
Alcaraz <i>et al.</i> (2011)	15 (15/0)	23 ± 3, trained	8	3	6 RM	NA	DEXA	Total LM: ↑
Antonio <i>et al.</i> (2014)	10 (8/2)	22 ± 3, resistance trained	8	NA	NA	Maintained habitual protein intake (~1.8 g·kg ⁻¹)	ADP	Total FFM: ↔
Antonio <i>et al.</i> (2015)	17 (13/4)	25 ± 7, resistance trained	8	5	NA	Maintained habitual protein intake (~2 g·kg ⁻¹)	ADP	Total FFM: ↑
Ara <i>et al.</i> (2006)	12 (12/0)	23 ± 2, recreationally active	6	3	50-90% 1RM	NA	DEXA	Total LM: ↔ Lower body LM: ↑

Avelar <i>et al.</i> (2019)	37 (37/0)	22 ± 3, untrained	6	3	NA	NA	DEXA	Lower body LM: ↔ Upper body LM: ↔ Trunk LM: ↔
Candow and Burke (2007)	15 (3/12)	43 ± 3, untrained	6	3	60-90% 1RM	NA	DEXA	Total LM: ↑
Coburn <i>et al.</i> (2006)	12 (12/0)	23 ± 3. untrained	8	3	80% 1RM	NA	UWW	Total FFM: ↔
Escalante <i>et al.</i> (2016)	10 (10/0)	26 ± 4, trained	8	3	75-85% 1RM	NA	DEXA	Total LM: ↑
Fyfe <i>et al.</i> (2016)	8 (8/0)	29 ± 6, recreationally active	8	3	65-90% 1RM	NA	DEXA	Total LM: ↔ Lower body LM: ↑ Upper body LM: ↔

Gobbo <i>et al.</i> (2013)	15 (15/0)	23 ± 4, untrained	8	4	6-12 RM	NA	DEXA	Total LM: ↑
Hoffman <i>et al.</i> (2012)	9 (9/0)	23 ± 2, resistance-trained	8	4	70% 1RM	NA	DEXA	Total LM: ↑
Moro <i>et al.</i> (2020)	9 (NA/NA)	22 ± 2, recreationally active	6	3	6 RM	NA	DEXA	Total LM: ↔
Paoli <i>et al.</i> (2017)	18 (18/0)	26 ± 4, recreationally active	8	3	6-8 RM	NA	DEXA	Total LM: ↑
Rozenek <i>et al.</i> (2002)	25 (25/0)	23 ± 5, untrained	8	4	70% 1RM	Supplement containing 450 g CHO, 24 g protein and 14 g fat. Half ingested during the day and the	UWW	Total FFM: ↑

						other half before bedtime		
Saremi <i>et al.</i> (2010)	8 (8/0)	22 ± 2, untrained	8	3	60-70% 1RM	NA	DEXA	Total LM: ↑
Scanlon <i>et al.</i> (2014)	13 (NA/NA)	71 ± 7, untrained	6	2	70-85% 1RM	NA	DEXA	Total LM: ↔
Slater <i>et al.</i> (2001)	17 (17/0)	24 ± 1, well- trained	6	2-3	3 RM	Supplement containing 24 g CHO, 42 g protein and 2 g fat	DEXA	Total LM: ↑
Spillane and Willoughby (2016)	11 (11/0)	21 ± 4, resistance- trained	8	4	70-80% 1RM	Supplement containing 196 g CHO, 94 g protein and 22 g fat. Half of the supplement was ingested prior to	DEXA	Total LM: ↔

						RE and the other half after RE		
Thorstensson <i>et al.</i> (1976)	14 (14/0)	Range: 19- 31, recreationally active	8	3	6 RM	NA	DEXA	Total LM: ↑
Tinsley <i>et al.</i> (2017)	10 (10/0)	21 ± 3, untrained	6	4	8-12 RM	NA	DEXA	Total LM: ↔
Weisgarber <i>et al.</i> (2012)	8 (4/5)	24 ± 4, untrained	8	4	6-10 RM	NA	DEXA	Total LM: ↔

ADP, air displacement plethysmography; CHO, carbohydrate; DEXA, dual energy X-ray energy absorptiometry; FFM, fat-free mass, LM, lean mass; RE, resistance exercise; UWW, underwater weighing; ↑, significant increase after RET; ↔, unchanged after RET; ↓, significant decrease after RET.

1.2.4 Effects of Resistance Exercise Training on Muscle Strength

Muscle strength is typically defined as the maximum force generating capacity of a muscle or muscle group (Kell *et al.*, 2001). Maintenance of muscle strength is crucial for retaining physical function, particularly with advanced neuromuscular ageing (Lavin *et al.*, 2019), to help attenuate risk of injuries arising from falls and subsequent loss of independence (Li *et al.*, 2018; Marzetti *et al.*, 2017; Mayer *et al.*, 2011). Because resistance exercise movements require substantial activation of motor units in active skeletal muscles (Sale, 1987), it is recognised as the most potent exercise modality for increasing muscle strength across all age groups. Indeed, muscle strength gains are observable after only several weeks of RET. In young adults, short-term RET has been shown to increase lower (Bell *et al.*, 2000; Brook *et al.*, 2015; de Souza *et al.*, 2013; Leger *et al.*, 2006; Moro *et al.*, 2020; Staron *et al.*, 1994; Stasinaki *et al.*, 2015) and upper (Bell *et al.*, 2000; Leger *et al.*, 2006; Moro *et al.*, 2020; Ogasawara *et al.*, 2011; Stasinaki *et al.*, 2015) body maximal isotonic muscle strength, measured by one-repetition maximum (1RM). Similarly, short-term RET increased lower (Chrusch *et al.*, 2001; Damush & Damush, 1999; Henwood & Taaffe, 2006; Pinto *et al.*, 2014; Scanlon *et al.*, 2014; Schlicht *et al.*, 2001) and upper (Daly *et al.*, 2013; Damush & Damush, 1999; Henwood & Taaffe, 2006) body maximal muscle strength in older adults. Compared to young and older adults, less is known regarding 1RM muscle strength responses to short-term RET in middle-aged adults.

In the few studies undertaken in middle-aged (~40 y) adults, 6-8 weeks of RET increases lower and upper body 1RM muscle strength by ~25-35% above pre-training levels after performing compound movements (e.g., leg press, leg extension, bench press, chest press) 2-4 times on non-consecutive days each week (Benton *et al.*, 2011; Candow & Burke, 2007; Izquierdo *et al.*, 2005). In contrast, Mikkola *et al.* (2012) reported a ~10% increase in leg press 1RM muscle strength after seven weeks of RET in middle-aged men. Factors that might explain the discrepant increases in the magnitudes of muscle strength from these studies in middle-aged

adults (Benton *et al.*, 2011; Candow & Burke, 2007; Izquierdo *et al.*, 2005; Mikkola *et al.*, 2012) may include differences in RET duration (six vs eight weeks) and the specific exercise tests used to assess lower (squat vs leg press) and upper (bench press vs chest press) body muscle strength. Regardless, it is apparent that short-term RET is capable of increasing whole body 1RM muscle strength in young and older adults with limited evidence suggesting that similar changes are achievable in middle-aged adults.

1.2.5 Resistance Exercise Training and Increased Protein Availability in Skeletal Muscle Anabolism

Nutrient availability is a key factor mediating exercise-induced skeletal muscle adaptations (Hawley *et al.*, 2011). Dietary protein ingestion, principally the essential amino acid leucine, increases rate of MPS (Moore *et al.*, 2015; Wilkinson *et al.*, 2013) and satellite cell activity (Dai *et al.*, 2015; Shamim *et al.*, 2018b). Post-exercise protein ingestion is crucial for skeletal muscle remodelling by stimulating rates of MPS through the transfer and incorporation of amino acids into skeletal muscle proteins (Stokes *et al.*, 2018). Indeed, net accretion of myofibrillar proteins can only be achieved through post-exercise protein feeding (Biolo *et al.*, 1997). While outside the scope of this review, it must be noted that several inter-related factors including dietary protein quantity, quality, source, food matrix in which protein is co-consumed and daily distribution of protein intake should be considered in order to optimise anabolic adaptation responses with RET (Burd *et al.*, 2019). However, the necessity for dietary protein intake to augment RET-induced gains in muscle mass compared to a placebo is equivocal with multiple meta-analyses reporting either beneficial or no positive effects on muscle growth and strength responses (Cermak *et al.*, 2012; Finger *et al.*, 2015; Hanach *et al.*, 2019; Messina *et al.*, 2018; Miller *et al.*, 2014; Morton *et al.*, 2017; Ten Haaf *et al.*, 2018; Valenzuela *et al.*, 2019).

One of the most comprehensive meta-analyses to examine the role of protein supplementation and RET-induced skeletal muscle anabolism, included 49 studies and 1,863 participants, and reported enhanced gains in muscle fiber size, fat-free mass and 1RM muscle strength with prolonged RET and increased protein intake compared to RET alone (i.e., without protein supplementation) (Morton *et al.*, 2017). Similar findings were reported in a meta-analysis by Cermak *et al.* (2012) where data from 22 studies and 680 participants revealed a positive effect of protein supplementation and prolonged RET for muscle fiber size, fat-free mass and 1RM leg press muscle strength. Whether protein supplementation is as efficacious for short-term RET remains to be determined. In any case, it has been suggested that the current recommended daily protein intake of $0.8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ is not sufficient for exercising adults (Jager *et al.*, 2017). Indeed, the International Society of Sports Nutrition Position Stand for ‘Protein and exercise’ stipulate that $\sim 1.4\text{-}2.0 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ of protein is required for promoting and building muscle mass (Jager *et al.*, 2017).

1.3 ENDURANCE EXERCISE TRAINING & SKELETAL MUSCLE ANABOLISM

1.3.1 Molecular Responses Implicated in Skeletal Muscle Anabolism and Catabolism with Endurance Exercise Training

Endurance exercise is characterised by continuous or intermittent movement (e.g., running, cycling, swimming) at submaximal intensities ($45\text{-}75\% \text{ VO}_{2\text{max}}$) for prolonged durations (>30 min). Endurance exercise training (ENT) increases $\text{VO}_{2\text{max}}$ which is primarily achieved by increases in skeletal muscle mitochondrial biogenesis and angiogenesis (Hawley *et al.*, 2014). The contractile stimulus and substrate perturbation induced by endurance exercise stimulates cell signalling cascades and transcriptional factors that modulate the expression of nuclear and mitochondrial genes encoding mitochondrial proteins (Lindholm *et al.*, 2014) (**Figure 1.4**). While the central focus of this review is directed toward anabolic-related adaptations with exercise training, a brief synopsis of the molecular mechanisms regulating mitochondrial-

related adaptations after ENT will be discussed. This will be followed by a summary of the literature relating to anabolic-centric gene and protein responses following endurance exercise in human skeletal muscle.

One so-called ‘master’ regulator of mitochondrial responses that has been intensely investigated following ENT is the peroxisome proliferator activated receptor γ coactivator-1 α (PGC-1 α). PGC-1 α is a transcriptional coactivator and stimulates mitochondrial biogenesis through recruitment and co-regulation of transcription factors that regulate skeletal muscle gene expression (Lin *et al.*, 2005). Interaction between PGC-1 α and nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) regulates the transcription of gene products involved in mitochondrial biogenesis. These mitochondrial biogenesis related genes include mitochondrial DNA (mtDNA) transcription, electron transport chain protein assembly and protein import machinery (PIM) complexes (Hood *et al.*, 2011a). NRF-1 also activates mitochondrial transcription factor (Tfam), an important regulator of mtDNA expression (Tryon *et al.*, 2015).

Increases in PGC-1 α mRNA levels have been consistently observed following endurance-based exercise in human skeletal muscle (Bartlett *et al.*, 2012; Edgett *et al.*, 2013; Lane *et al.*, 2015; Perry *et al.*, 2010), with the highest mRNA abundance generally observed in the first 2-4 h post-exercise. Egan *et al.* (2010) showed that increased PGC-1 α mRNA abundance in skeletal muscle may also be dependent on the type and intensity of endurance-based exercise with larger increases in PGC-1 α mRNA observed following a short-duration, high-intensity ($\sim 80\%$ $\text{VO}_{2\text{peak}}$) bout of cycling compared to an energy-matched session of a prolonged duration, low-intensity (40% $\text{VO}_{2\text{peak}}$). The authors attributed this increase in PGC-1 α to a greater upstream phosphorylation of the AMP-activated protein kinase (AMPK), calcium/calmodulin-dependent protein kinase II (CaMKII) and p38 mitogen-activated protein kinase (p38 MAPK). Furthermore, six weeks of moderate intensity ENT (60% $\text{VO}_{2\text{max}}$) can induce two-fold increases in PGC-1 α mRNA expression (Russell *et al.*, 2003). Previous work has also shown that PGC-1 α can translocate to the nucleus in following both moderate (Little

et al., 2010a) and high-intensity (Little *et al.*, 2011b) endurance exercise, while its nuclear abundance (Little *et al.*, 2010b) and total protein levels (Gurd *et al.*, 2010) are increased after just two weeks of interval-based ENT. PGC-1 α can also localise to mitochondria following endurance exercise where it forms a transcriptional complex with Tfam (Safdar *et al.*, 2011).

Accumulating evidence also indicates the tumour suppressor protein p53 can regulate protein targets involved in mitochondrial biogenesis and oxidative metabolism processes (Smiles & Camera, 2018). In this regard, Tachtsis *et al.* (2016) demonstrated increases in nuclear p53 abundance following 60 min of continuous cycling exercise in untrained individuals, indicating a propensity for p53 to initiate mitochondrial biogenesis and remodelling processes. Taken together, endurance exercise stimulates cell signalling pathways that promote molecular events that are important to stimulate mitochondrial biogenesis. Important regulators of exercise-induced mitochondrial biogenesis such as PGC-1 α and p53 are central mediators to this response with endurance exercise.

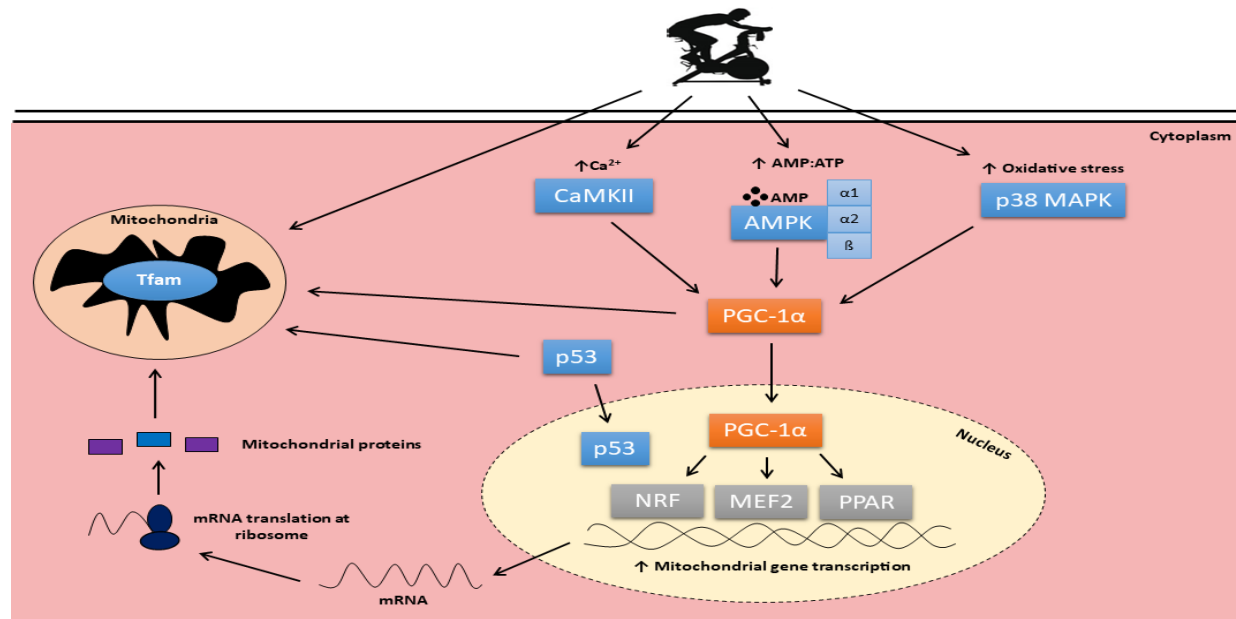


Figure 1.4 Simplified schematic of signalling events induced by aerobic-based exercise in skeletal muscle.

AMPK, adenosine monophosphate-dependent protein kinase; CaMKII, calcium-calmodulin-dependent protein kinase II; MEF, myocyte enhancer factor; mRNA, messenger ribonucleic acid; NRF, nuclear respiratory factor; p38 MAPK, p38 mitogen-activated protein kinase; p53, tumour protein p53; PGC-1 α , peroxisome proliferator activated receptor γ coactivator-1 α ; PPAR, peroxisome proliferator-activated receptor; Tfam, mitochondrial transcription factor A.

The capacity for endurance exercise training to promote skeletal muscle growth, and how this compares to resistance exercise, has gained interest over the past decade among exercise scientists (Grgic *et al.*, 2019; Konopka & Harber, 2014). Similar to RET, most investigations of the potency of endurance exercise to upregulate the molecular machinery governing skeletal muscle anabolism have centred on the mTORC1-signalling cascade (**Figure 1.5**). Endurance-based exercise (~45-60 min at ~70-75% $\text{VO}_{2\text{max}}$) has been shown to increase post-exercise mixed muscle protein synthesis in young (Harber *et al.*, 2009a; Harber *et al.*, 2010; Miller *et al.*, 2005) and older adults (Durham *et al.*, 2010). Increases in $\text{Akt}^{\text{Ser473}}$, $\text{mTOR}^{\text{Ser2448}}$ and $\text{p70S6K}^{\text{Thr389}}$ phosphorylation have also been reported in the acute recovery period following one-legged cycling (60 min at 65-70% $\text{VO}_{2\text{max}}$) in young men (Mascher *et al.*, 2011). In contrast, Breen *et al.* (2011) observed no change in rates of mitochondrial or myofibrillar protein synthesis, or $\text{mTOR}^{\text{Ser2448}}$ and $\text{p70S6K}^{\text{Thr389}}$ phosphorylation, after one bout of continuous cycling (90 min at ~75% $\text{VO}_{2\text{max}}$) in well-trained men. Similar findings have also been reported in well-trained adults (Coffey *et al.*, 2006) and recreationally active young men (Camera *et al.*, 2010) following a single bout of cycling exercise (60 min at ~70% $\text{VO}_{2\text{peak}}$) where, despite an increase in $\text{Akt}^{\text{Ser473}}$ phosphorylation, no increase in $\text{p70S6K}^{\text{Thr389}}$ phosphorylation was observed during the an acute recovery period (0-3 h).

After 10 weeks of ENT (30 min at 75% $\text{VO}_{2\text{peak}}$), Wilkinson *et al.* (2008) demonstrated elevated rates of mitochondrial, but not myofibrillar, protein synthesis in the acute recovery period following a single post-intervention bout of endurance exercise in untrained young men. Phosphorylation status of $\text{mTOR}^{\text{Ser2448}}$ and $\text{p70S6K}^{\text{Thr389}}$ increased immediately post-endurance exercise but returned to resting levels four hours later, before and after 10 weeks of ENT. Moreover, there was no change in basal phosphorylation levels of any signalling proteins after 10 weeks of ENT (Wilkinson *et al.*, 2008). Collectively, and similar to resistance exercise, endurance exercise stimulates translational signalling proteins in during short-term (i.e., several hours) recovery. As such, the capacity to ‘switch on’ such translational machinery does not

appear to be divergent between resistance and endurance-based exercise in human skeletal muscle and likely indicates increases in translation initiation signalling over time with ENT are mainly directed toward the synthesis of mitochondrial proteins. Indeed, aerobic exercise training-induced increases in MPS can stimulate mitochondrial protein synthesis rather than only increased muscle cell size.

With regard to muscle proteolytic markers, after a single bout of endurance exercise (45 min at $\sim 75\%$ $\text{VO}_{2\text{max}}$) in young men, there is a decrease myostatin mRNA, a key inhibitor of muscle growth and activator of atrophy proteins (Lee, 2004) in the acute post-exercise recovery period (Harber *et al.*, 2009a; Harber *et al.*, 2010) and following 12 weeks of ENT (~ 30 -45 min at 60-80% HRR) in older women (Konopka *et al.*, 2010). Ubiquitin proteasome pathway markers such as forkhead box O3 (FOXO3a), MuRF-1 and atrogin-1 have also been measured following acute endurance exercise. Increased MuRF-1 mRNA in the acute recovery period has been observed following cycling exercise (~ 60 mins at $\sim 70\%$ $\text{VO}_{2\text{max}}$) in recreationally active men (Harber *et al.*, 2009a; Harber *et al.*, 2010). In contrast, FOXO3a and atrogin-1 mRNA abundance are unchanged following a single bout of endurance exercise (Harber *et al.*, 2009a; Harber *et al.*, 2010).

Less is known about changes in muscle proteolytic markers following short-term or prolonged ENT. Ten weeks of ENT increased MuRF-1 and atrogin-1 mRNA, but not total protein content, in young untrained men (Stefanetti *et al.*, 2015). A 12-week study in older women performing continuous cycling exercise training showed decreased mRNA expression of myostatin but not MuRF-1 or atrogin-1 (Konopka *et al.*, 2010). While acute investigations indicate that key markers of protein degradation are downregulated following endurance exercise, further work following short-term and prolonged ENT is needed to understand how alterations in markers of protein degradation may impact net protein turnover and subsequent changes in the accrual of lean mass.

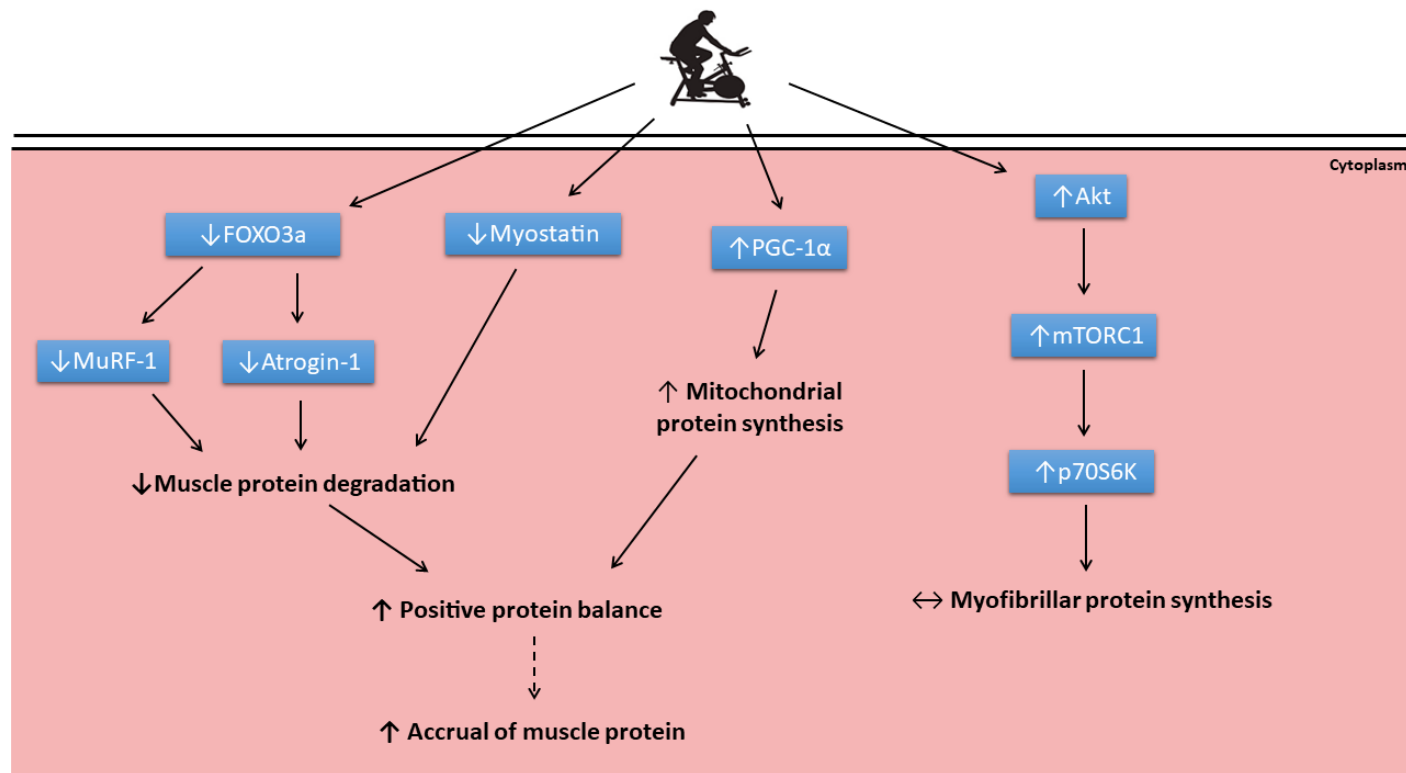


Figure 1.5 Simplified schematic of signalling events that may underpin skeletal muscle growth with aerobic-style exercise training.

Adapted from Konopka and Harber (2014). Akt, protein kinase B; atrogin-1, muscle atrophy F-box; mTORC1, FOXO3a, forkhead box O3; MuRF-1, muscle RING-finger protein-1; mammalian target of rapamycin complex 1; PGC-1 α , peroxisome proliferator activated receptor γ coactivator-1 α ; p70S6K, ribosomal protein S6 kinase beta-1. Dotted line indicates proposed mechanism explaining, in part, increased muscle mass with endurance exercise training.

1.3.2 Effects of Endurance Exercise Training on Skeletal Muscle Fiber Cross-Sectional Area

Compared to RET, few studies have investigated changes in skeletal muscle fiber size following endurance-based exercise in human skeletal muscle. Intuitively, this likely relates to ENT not traditionally being associated as an ‘anabolic’ stimulus. However, prolonged (i.e., ≥ 12 wk) cycling ENT induces increases in skeletal muscle fiber size in young (Fry *et al.*, 2014; Harber *et al.*, 2012) and older adults (Charifi *et al.*, 2003; Fry *et al.*, 2014; Harber *et al.*, 2009b). In contrast, Kraemer *et al.* (1995) observed a decrease in skeletal muscle fiber size following 12 weeks of high-intensity continuous and interval-style running ENT ($>80\%$ $\text{VO}_{2\text{max}}$) in young adults. Moreover, studies in young (Bell *et al.*, 2000; Nelson *et al.*, 1990), middle-aged (Karavirta *et al.*, 2011) and older adults (Sipilä *et al.*, 1997) report no change in skeletal muscle fiber size following prolonged ENT. Such disparity in muscle fiber CSA responses may, in part, be due to differences in the age of participants ($\sim 20\text{-}30$ y vs $\sim 65\text{-}75$ y) and ENT mode used (running vs cycling, eccentric versus concentric loading). Fewer studies have examined muscle fiber CSA changes following short-term training. In response to 8-10 weeks of ENT, skeletal muscle fiber CSA was unchanged in young (de Souza *et al.*, 2014; Farup *et al.*, 2012; McCarthy *et al.*, 2002) and older adults (Hepple *et al.*, 1997). Similar findings have been reported after six weeks of cycling ENT in young adults (Howald *et al.*, 1985; Joannis *et al.*, 2015). While such findings may indicate short-term ENT is unable to induce skeletal muscle fiber hypertrophy, it is possible the frequency and intensity of exercise training programs employed may have been below a ‘threshold stimulus’ needed to increase fiber CSA. Moreover, no studies have investigated the capacity for short-term ENT to increase skeletal muscle fiber size in middle-aged adults.

1.3.3 Effects of Endurance Exercise Training on Body Composition and Whole-Muscle Morphology

Several studies have investigated changes in lean/fat-free mass following short-term ENT, with most showing no change post-intervention (**Table 1.4**). Of the studies that have measured increases in lean/fat-free mass, Shamim *et al.* (2018a) observed an increase in total lean mass of ~1 kg after eight weeks of cycling ENT in recreationally active young men, likely facilitated by the consumption of a high protein diet (~2 g·kg⁻¹·day⁻¹) consumed by participants throughout the intervention. Lean/fat-free mass has also been shown to increase by ~0.5 kg following short-term running (Macpherson *et al.*, 2011) and cycling (Matsuo *et al.*, 2014b) ENT in young men. In extension of these increases in lean/fat-free mass, work from the Trappe laboratory reported increased *quadriceps femoris* volume (via MRI) after 12 weeks of ENT in young men and older adults (Harber *et al.*, 2009b; Harber *et al.*, 2012). In a short-term study, McPhee *et al.* (2010) reported increased *quadriceps femoris* volume (via MRI) following six weeks of ENT in young women. Shamim *et al.* (2018a) observed increased *vastus lateralis* muscle thickness at week four and eight of a 12-week cycling ENT programme in young men. Collectively, short-term and prolonged ENT can increase estimates of whole-muscle size although most studies report no change in lean/fat-free mass following short-term ENT.

It has been suggested that higher frequency ENT (~4-5 sessions per week) may be necessary to induce skeletal muscle hypertrophy compared to ENT programming involving exercise sessions on two to three non-consecutive days per week (Konopka & Harber, 2014). As most short-term ENT studies have incorporated protocols in which there are only two to three sessions per week (**Table 3**), this may, in part, explain the lack of increase in lean/fat-free mass reported after short-term ENT. In this regard, total fat-free mass was shown to be unchanged after six weeks of cycling ENT performed three non-consecutive days per week in young men despite increases in, lower body lean mass (Higgins *et al.*, 2016). Another caveat arising from the studies included in **Table 3** is that few included a nutritional intervention,

particularly protein supplementation, to help augment skeletal muscle growth responses. Regardless, further work investigating lean/fat-free mass and muscle morphology responses after short-term ENT in middle-aged adults is required. Indeed, retention of lean mass is critical for middle-aged adults more prone to accelerated skeletal muscle loss with advancing age.

Table 1.4 Studies assessing lean mass or fat-free mass after short-term ENT.

Study	Total <i>N</i> in ENT group (male/female)	Age (years), training status	Intervention duration (weeks)	ENT frequency (days·week ⁻¹)	Mode	Intensity of training	Nutrition intervention	Lean/fat-free mass measurement	Main findings after short-term ENT
Higgins <i>et al.</i> (2016)	29 (0/29)	20 ± 2, untrained	6	3	Cycling	60-70% HRR	NA	DEXA	Total FFM: ↔ Lower body FFM: ↑
Hwang <i>et al.</i> (2016)	14 (7/7)	66 ± 2, untrained	8	4	Arm and leg non-weightbearing ergometry	70% HR _{peak}	NA	DEXA	Total FFM: ↔
Knuiman <i>et al.</i> (2019)	21 (21/0)	23 ± 1, recreationally active	10 Measurements also taken at week 5	3	Cycling	~70% HRR	Beverage containing 26.3 g CHO, 0.6 g casein and 2.4 g fat. Consumed after each	DEXA	Total LM after week 5: ↔

							training session and every night before bed		
Kong <i>et al.</i> (2016)	8 (0/8)	20 ± 2, untrained	5	3	Cycling	65% VO _{2peak}	NA	DEXA	Total FFM: ↔
Macpherson <i>et al.</i> (2011)	10 (NA/NA)	23 ± 3, recreationally active	6	3	Running	65% VO _{2max}	NA	ADP	Total LM: ↑
Matsuo <i>et al.</i> (2014a)	12 (12/0)	29 ± 8, untrained	8	3	Cycling	60-65% VO _{2peak}	NA	DEXA	Total FFM: ↔
Matsuo <i>et al.</i> (2014b)	14 (14/0)	26 ± 6, untrained	8	5	Cycling	60-65% VO _{2peak}	NA	DEXA	Total FFM: ↑
Matsuo <i>et al.</i> (2015)	13 (13/0)	47 ± 8, untrained	8	3	Cycling	60-65% VO _{2peak}	NA	DEXA	Total FFM: ↔

Shamim <i>et al.</i> (2018a)	10 (10/0)	24 ± 5, recreationally active	12 Measuremen ts also taken at week 4 and 8	3	Cycling	50-70% MAP	High protein diet (~2 g·kg ⁻¹ ·day ⁻¹)	DEXA	LM at week 4 and 8: ↑
Sawyer <i>et al.</i> (2016)	9 (4/5)	35 ± 8, untrained	8	3	Cycling	70-75% HR _{max}	NA	DEXA	Total FFM: ↔

ADR, air displacement plethysmography; DEXA, dual energy X-ray energy absorptiometry; FFM, fat-free mass; HR_{max}, maximum heart rate; HR_{peak}, peak heart rate; HRR, heart rate reserve; LM, lean mass; MAP, maximal aerobic power; UWW, underwater weighing; VO₂ peak; peak oxygen uptake; ↑, significant increase after ENT; ↔, unchanged after ENT; ↓, significant decrease after ENT.

1.3.4 Effects of Endurance Exercise Training on Muscle Strength

Typically, endurance-based exercise is not been associated with increases in muscle strength. This premise is largely due to early observations of inferior gains in strength compared to RET and/or concurrent resistance and endurance exercise training in recreationally active young adults (Dolezal & Potteiger, 1998; Hickson, 1980; Kraemer *et al.*, 1995; McCarthy *et al.*, 2002). However, several studies in older adults show prolonged ENT increases lower body muscle strength (Coggan *et al.*, 1992; Harber *et al.*, 2009b; Markofski *et al.*, 2019) suggesting that exercise such as cycling, running and even walking, may result in skeletal muscle adaptations beyond traditional measures of enhanced cardiorespiratory fitness. Indeed, adults of all ages regularly performing ENT for at least ~10 y exhibit superior muscle strength compared to age-matched sedentary counterparts (Crane *et al.*, 2013). Compared to prolonged ENT, less is known regarding short-term ENT and muscle strength responses. It is important to assess muscle strength after short-term ENT programmes in all age groups to determine whether ‘aerobic exercise’ can simultaneously improve cardiorespiratory fitness and muscle strength. Such improvements may be of particular relevance for populations who are highly sedentary/deconditioned such as middle-aged adults with limited exercise training time (e.g., prior to planned surgery).

Of the few studies that have investigated changes in muscle strength in middle-aged adults following short-term ENT, Izquierdo *et al.* (2005) assessed lower and upper body 1RM muscle strength before and after eight weeks of ENT (~30-40 min at 55-85% maximal power output (W_{max})) performed two days per week in middle-aged men. In that study, ENT increased half-squat but not bench press 1RM muscle strength (Izquierdo *et al.*, 2005). Conversely, Mikkola *et al.* (2012) reported no change in leg extension 1RM muscle strength following six weeks of ENT (30 min at below aerobic threshold) performed twice weekly in middle-aged men. In summary, limited information is available regarding short-term muscle strength responses following ENT in middle-aged adults. Further work is warranted due to potential

concurrent improvements in aerobic capacity and muscle strength particularly where improvements in physical function are highly desirable in limited time (e.g., peri-operative period).

1.3.5 Endurance Exercise Training and Increased Protein Availability

Due to the inherently high metabolic demands of endurance exercise, post-exercise nutrient recovery strategies have primarily focused on the role of carbohydrate ingestion to primarily replenish skeletal muscle glycogen stores (Burke *et al.*, 2017). However, endurance exercise stimulates protein breakdown (Howarth *et al.*, 2010; Lemon & Mullin, 1980) and the need for synthesis of enzymes involved in substrate metabolism and oxygen transport (Tarnopolsky, 2004). As such, protein ingestion following endurance exercise is an important component of recovery to support and maximise skeletal muscle adaptive responses to training (Moore *et al.*, 2014).

The majority of investigations to date have determined the role of protein supplementation in augmenting anabolic responses to RET (Cermak *et al.*, 2012; Morton *et al.*, 2017). Comparatively, less is known about the role of increased protein availability in eliciting enhanced gains in skeletal muscle mass/size and strength following ENT. Results from acute investigations into protein ingestion and endurance exercise (~60-90 min at ~60-70% $\text{VO}_{2\text{peak}}$) have revealed several findings supporting a role for increased protein availability to enhance skeletal muscle adaptations. Key findings from such studies include increases in mixed muscle protein synthesis in recreationally active men following 2 h cycling (Howarth *et al.*, 2009) and myofibrillar protein synthesis in well-trained men after either 60 min running (70% $\text{VO}_{2\text{peak}}$) (Abou Sawan *et al.*, 2018) or 90 min cycling (~60% W_{max}) (Churchward-Venne *et al.*, 2020). Pre-exercise ingestion of a protein-rich beverage containing 5 g of leucine increased rates of myofibrillar protein synthesis and mTORC^{Ser2448} and p70S6K^{Thr389} phosphorylation following 100 min cycling in well-trained men (Rowlands *et al.*, 2015). The translation of such acute

increases in muscle protein synthesis to actual changes in lean body mass following ENT has received less attention.

Knuiman *et al.* (2019) reported that protein supplementation (~29 g casein provided after every exercise session) increased lean mass to a greater extent than a non-protein supplemented group (~1.5 kg vs 0.3 kg) in recreationally young men after 10 weeks of cycling ENT (60 min at vigorous intensity, three days per week), despite similar improvements in maximal aerobic capacity. Jonvik *et al.* (2019) reported increased leg lean mass, but not total lean mass, with protein supplementation (~29 g casein provided after every exercise session) after 12 weeks of cycling ENT (three days per week) incorporating interval and steady-state sessions in recreationally active young men. While both of these studies provided a protein supplement post-exercise and before sleep to maximise MPS responses, greater gains in total and/or leg lean mass may have been possible through greater daily intakes of protein or strategic distribution of protein throughout the day (Areta *et al.*, 2013) to maximally stimulate MPS rates (Churchward-Venne *et al.*, 2012). In any case, results from the aforementioned studies (Jonvik *et al.*, 2019; Knuiman *et al.*, 2019) provide evidence that short-term and prolonged ENT can increase lean mass in young adults.

1.4 HIGH-INTENSITY INTERVAL TRAINING & SKELETAL MUSCLE ANABOLISM

1.4.1 Introduction to the Potential Role of HIIT in Skeletal Muscle Mass Maintenance

Interval training, such as high-intensity interval training (HIIT) and sprint interval training (SIT), has gained in popularity in recent times (Gibala, 2018; Thompson, 2018), and may be a viable, yet overlooked alternative to RET for promoting muscle mass accrual. HIIT programming is infinitely variable, but typically characterised by brief periods (≤ 4 min) of intense continuous exercise (80-100% peak heart rate [HR_{peak}]) interspersed with short periods of rest or recovery. In contrast, SIT involves shorter (≤ 30 s) ‘all-out’ work periods performed at $\geq 100\%$ of the power output/speed that elicits an individual’s VO_{2max} (Weston *et al.*, 2014).

The enhanced metabolic and cardiorespiratory effects of HIIT and SIT in skeletal muscle have been well documented (Gibala, 2009; Gibala *et al.*, 2012; MacInnis & Gibala, 2017). However, much less is known about the impact of aerobic-based HIIT and SIT modalities (i.e., cycling, running, swimming) on muscle growth responses and whether these interventions can promote gains in muscle hypertrophy, lean mass and strength, particularly when undertaken with optimal nutritional support. Furthermore, it is of interest to exercise physiologists, clinicians and the general population alike to determine if HIIT can simultaneously maintain and/or improve cardiorespiratory fitness and muscle mass, given the reduced time commitment compared to traditional resistance or aerobic exercise modalities. Additionally, RET participation rates remain low among older adults (Bennie *et al.*, 2016; Humphries *et al.*, 2010; Lin *et al.*, 2018; Merom *et al.*, 2012), in part due to a perceived need for specialised equipment and correct technique to prevent injury, thus calling for alternative strategies to complement or substitute traditional strength-based exercise training.

Various HIIT and SIT protocols in both healthy and clinical populations have been shown to improve cardiometabolic health outcomes (Batacan *et al.*, 2017; Cassidy *et al.*, 2017; Weston *et al.*, 2014). Some of the more popular and well researched protocols are the ‘Norwegian’ (Moholdt *et al.*, 2009; Robinson *et al.*, 2017; Rognmo *et al.*, 2004; Wisloff *et al.*, 2007; Wyckelsma *et al.*, 2017), ‘Gibala’ (Blue *et al.*, 2017; Hood *et al.*, 2011b; Joannis *et al.*, 2013; Little *et al.*, 2011a) and ‘Tabata’ models (Joannis *et al.*, 2015; Scribbans *et al.*, 2014; Tabata *et al.*, 1996), as well as Wingate-based training (Burgomaster *et al.*, 2008; Gibala *et al.*, 2006; Granata *et al.*, 2016; Scalzo *et al.*, 2014) and reduced exertion HIIT (REHIT) (Gillen *et al.*, 2016; Metcalfe *et al.*, 2012; Metcalfe *et al.*, 2016) (**Table 1.5**). Importantly, various interval training regimes (Hwang *et al.*, 2016; Mejías-Peña *et al.*, 2016; Sogaard *et al.*, 2017), particularly the ‘Norwegian’ model (Kim *et al.*, 2017; Kovacevic *et al.*, 2020; Robinson *et al.*, 2017; Stensvold *et al.*, 2020; Wisloff *et al.*, 2007), have been shown to increase aerobic fitness in older adults. HIIT improves skeletal muscle oxidative capacity, primarily via activation of

signalling cascades that stimulate mitochondrial biogenesis and angiogenesis (Gibala *et al.*, 2012). SIT and ENT can induce similar improvements in oxidative metabolism with vastly different training volumes (Burgomaster *et al.*, 2008; Gillen *et al.*, 2016). As such, interval training is often considered a time-efficient alternative to traditional aerobic (endurance) exercise training.

Table 1.5 Popular high-intensity interval training protocols.

Model	Interval training protocol		
	Repetitions	Work (duration [s], intensity)	Rest (s)
REHIT	2-3	20 s, ‘all out’ sprint	120-180
Tabata	7-8	20 s, ~170% VO _{2max}	10
Wingate	4-10	30 s, ‘all out’ sprint	240
Gibala	10	60 s, >90% HR _{peak}	60
Norwegian	4	240 s, 85-95% HR _{peak}	180

HR_{peak}, peak heart rate; REHIT, reduced exertion high-intensity interval training; maximal oxygen uptake, VO_{2max}.

Despite similarities in skeletal muscle oxidative capacity and subjective ratings of enjoyment between HIIT and ENT (Oliveira *et al.*, 2018), two weeks of HIIT induces a different neuromuscular profile to ENT in young men (Martinez-Valdes *et al.*, 2017; MARTINEZ-VALDES *et al.*, 2018). Using high-density electromyography (EMG), HIIT has been shown to increase muscle fiber conduction velocity (MARTINEZ-VALDES *et al.*, 2018), maximal knee extensor torque and discharge rate of high-threshold motor units (Martinez-Valdes *et al.*, 2017), all factors related to maximal force production. In contrast, only minor changes in these functional measures have been observed after ENT. There is a greater activation of type II muscle fibers with increasing exercise intensity/contractile force (Edgett *et al.*, 2013; Krstrup

et al., 2004), with these fibers having the greatest potential for hypertrophy following RET (Folland & Williams, 2007). Considering that changes to neural factors and muscle fiber size following RET are directly linked to maximal strength gains (Duchateau *et al.*, 2006), HIIT may also increase muscle strength, albeit to a lesser magnitude than RET, particularly in populations sensitive to relatively intense exercise (e.g., untrained/ageing populations). While the results from these studies (Martinez-Valdes *et al.*, 2017; MARTINEZ-VALDES *et al.*, 2018) provide information regarding neuromuscular adaptations with HIIT, the focus of the following sections of this review is on the capacity for HIIT to increase skeletal muscle mass and size as a practical strategy to offset the inevitable loss in muscle mass with advancing age.

1.4.2 Molecular Responses Implicated in Skeletal Muscle Anabolism and Catabolism with HIIT

In response to skeletal muscle contraction, a multitude of cellular events are initiated that modulate the expression of specific gene sets that encode proteins that ultimately form the basis of adaptation responses (Camera *et al.*, 2016). The majority of studies investigating molecular responses to HIIT have focused on pathways regulating mitochondrial biogenesis and insulin sensitivity in skeletal muscle (Gibala *et al.*, 2012). Recent studies incorporating high-throughput ‘-omics’ techniques have explored the ‘global’ effects of HIIT at the transcriptional and translational levels. Key findings from these studies reveal unique molecular ‘signatures’ supporting the notion that genes and proteins implicated in muscle mass regulation are up-regulated through interval-style exercise (**Table 1.6**).

Table 1.6 Studies assessing molecular changes linked to muscle growth in response to HIIT/SIT.

Study	Total N in interval training group (male/female)	Study groups	Age (y)	Population	Intervention duration	Nutrition intervention	Modality	Sets	Work	Rest	Measurements related to muscle growth responses	Main findings regarding HIIT and muscle growth
<i>Acute (single bout)</i>												
Bell <i>et al.</i> (2015)	7 (7/0)	HIIT vs ENT vs RET	67 ± 5	Older sedentary men, untrained	One bout	NA	Cycling	5	120 s, 80-100% PPO	60 s	Myofibrillar and sarcoplasmic PS	Myofibrillar PS: ↑ at 24 and 48 h, sarcoplasmic PS: ↑ at 24 h, returned to baseline at 48 h
Esbjörnsson <i>et al.</i> (2012)	17 (9/8)	SIT	Males: 26 ± 4 Females: 25 ± 2	Healthy young adults, recreationally active	One bout	NA	Cycling	3	30 s, 'all-out' sprint	20 min	Protein expression	↑ p-Akt, p-mTOR, p-p70S6K and p-rpS6
Rundqvist <i>et al.</i> (2019)	14 (7/7)	SIT	Males: 26 ± 4 Females: 25 ± 2	Healthy young adults, recreationally active	One bout	NA	Cycling	3	30 s, 'all-out' sprint	20 min	Transcriptome	↑ <i>FZD7</i> , <i>MYOD1</i> , ↓ <i>MSTN</i>
Coffey <i>et al.</i> (2011)	8 (8/0)	SIT + nutrients vs SIT	21 ± 3	Healthy young men, recreationally active	One bout	Pre exercise beverage containing 24 g whey, 4.8 g leucine, 50 g maltodextrin	Cycling	10	6 s, 0.75 N m torque·kg ⁻¹ BM	60 s	Myofibrillar and mitochondrial PS, protein expression	Myofibrillar PS: ↑ with SIT + nutrients, ↔ with SIT, mitochondrial PS: ↔ with either

												condition, p-Akt and p-mTOR: ↑ only with HIIT + nutrients
Rundqvist <i>et al.</i> (2017)	12 (9/3)	SIT + nutrients vs SIT	26 ± 4	Healthy young adults, recreationally active	One bout	Pre exercise beverage containing 300 mg·kg ⁻¹ EAA, 1 g·kg ⁻¹ maltodextrin	Cycling	3	30 s, 'all-out' sprint	20 min	Mixed MPS, signalling responses	SIT + nutrients: ↑ SNAT2 mRNA and protein expression, ↑ Akt/mTOR activity and likely ↑ MPS. SIT without nutrients did not increase these responses.
<i>Short-term (several weeks to months)</i>												
Scalzo <i>et al.</i> (2014)	21 (11/10)	HIIT (males vs females)	23 ± 3	Healthy young men & women, recreationally active	3 wk	NA	Cycling	4-8	30 s, resistance of 7.5% BM	240 s	Mixed, cytosolic and mitochondrial PS, protein expression	Males > females for all protein fractions
Miyamoto-Mikami <i>et al.</i> (2018)	11 (11/0)	SIT	23 ± 3	Healthy young men, recreationally active	6 wk	NA	Cycling	6-7	20 s, 170% VO _{2peak}	10 s	Transcriptome, protein expression	↑ <i>CARNS1</i> , <i>MYLK4</i> , <i>PPP1R3C</i> , <i>SGK1</i> and <i>PPARGC1A</i> mRNA and protein

Joannisse <i>et al.</i> (2013)	15 (0/15)	HIIT	27 ± 8	Healthy young women, untrained	6 wk	NA	Cycling	10	60 s, ~90% HR _{peak}	60 s	Muscle fiber CSA	Muscle fiber CSA: ↔
Joannisse <i>et al.</i> (2015)	10 (NA/NA)	SIT vs ENT	21 ± 2	Young adults, recreationally active	6 wk	NA	Cycling	8	20 s, ~170% VO _{2peak}	10 s	Muscle fiber CSA	Muscle fiber CSA: ↔
Joannisse <i>et al.</i> (2015)	14 (7/7)	HIIT	29 ± 9	Overweight and obese adults, untrained	6 wk	NA	Cycling	3	20 s, 'all out' sprint	120 s	Muscle fiber CSA	Muscle fiber CSA: ↔
<i>Prolonged (several months)</i>												
Leuchtmann <i>et al.</i> (2020)	9 (9/0)	HIIT vs RET	67 ± 4	Older recreationally active men	12 wk	Post exercise beverage containing 30 g whey protein	Cycling	7	60 s, 85% PPO	240 s	Muscle fiber CSA	Muscle fiber CSA: ↔
Robinson <i>et al.</i> (2017)	Young: 14 (7/7) Old: 9 (4/5)	HIIT vs RET vs CT (for young and old)	Young 18-30, Old: 65-80	Young and old adults, untrained	12 wk	NA	Cycling	4	240 s, >90% VO _{2peak}	180 s	Mitochondrial PS, transcriptome, proteome	Young and old: ↑ mitochondrial ribosome protein abundance, ↑ mitochondrial PS

BM, body mass; CSA, cross-sectional area; CT, combined training; EAA, essential amino acids; HIIT, high-intensity interval training; HR_{peak}, peak heart rate; ENT, moderate intensity continuous training; MPS, muscle protein synthesis; N m; Newton metres; PPO, peak power output; PS, protein

synthesis; RET, resistance exercise training; SC, satellite cell; SIT, sprint interval training; $\text{VO}_{2\text{peak}}$, peak oxygen uptake; wk, weeks; $\uparrow$, significant increase with HIIT, $\leftrightarrow$, unchanged with HIIT, $\downarrow$, significant decrease with HIIT.

Rundqvist *et al.* (2019) were among the first to examine the global gene expression profiles in human skeletal muscle (*vastus lateralis*) from young (~26 y) adults in response to a single bout of sprint cycling exercise (3 x 30 s ‘all-out’ sprints). A biopsy obtained ~2 h following the final sprint revealed differential expression of 879 genes (471 genes upregulated, 408 genes downregulated). Notably, sprint exercise significantly increased expression of genes implicated in the regulation of muscle mass including frizzled class receptor 7 (*FZD7*) and myogenic differentiation 1 (*MYOD1*), while concomitantly downregulating myostatin (*MSTN*) expression, a key suppressor of skeletal muscle growth. However, the acute nature of the sprint exercise protocol makes it difficult to assess the contribution and involvement of the collective changes in these gene transcripts to promote the requisite molecular signals for muscle hypertrophy responses.

Extending these findings, Miyamoto-Mikami *et al.* (2018) examined global gene expression profiles (*vastus lateralis*) following six weeks of Tabata-style SIT (20 s x 6-7 reps at 170% $\text{VO}_{2\text{max}}$) in healthy young (~23 y) men. These workers reported 79 genes were upregulated and 73 genes downregulated post-intervention (Miyamoto-Mikami *et al.*, 2018). Given the transient nature of many exercise-sensitive transcripts, and the time-course of sampling of the post-exercise training muscle biopsy (48–72 h after the last training session), it is likely that changes in expression of many transcripts may have been missed. Nonetheless, the CSA of the *quadriceps femoris* and hamstring muscles (MRI) were both increased following SIT, despite downregulation of several genes such as myosin heavy chain 1 (*MYH1*), myosin light chain kinase 2 (*MYLK2*) and nebulin related anchoring protein (*NRAP*) (Miyamoto-Mikami *et al.*, 2018), genes that encode proteins with putative roles in contractile function. Furthermore, gene expression of myostatin was decreased following HIIT (Miyamoto-Mikami *et al.*, 2018). In addition to changes at the mRNA level, the authors also observed increased protein expression of carnosine synthase 1 (*CARNS1*), myosin light chain kinase family member 4 (*MYLK4*), protein phosphatase 1 regulatory subunit 3 C (*PPP1R3C*), serum/glucocorticoid regulated

kinase 1 (*SGK1*) and peroxisome proliferator activated receptor gamma (*PPARGC1A*) (Miyamoto-Mikami *et al.*, 2018). *CARNS1* and *MYLK4* are involved in events that may improve force generating capacity. Carnosine synthase, an enzyme catalysing the β -alanyl-L-histidine dipeptide to carnosine encoded by the *CARNS1* gene, improves pH buffering capacity and increases calcium (Ca^{2+}) sensitivity to the contractile apparatus (Dutka *et al.*, 2012). Skeletal muscle myosin light chain kinase, a Ca^{2+} /calmodulin-dependent protein kinase encoded by the *MYLK* gene, phosphorylates the regulatory light chain of myosin in the sarcomere providing mechanical support during force generation (Kamm & Stull, 2001). Collectively, these data provide preliminary evidence for enhanced calcium handling that may support improved force generating capacity and possibly muscle hypertrophy following SIT. Indeed, contraction-induced alterations in intracellular $[\text{Ca}^{2+}]$ may be linked to distinctive programs of gene expression that establish phenotypic diversity among skeletal muscle fibers and confer some of the whole-body adaptations after SIT protocols (Gibala & Hawley, 2017).

Recently, Robinson *et al.* (2017) compared transcriptome and proteome responses from skeletal muscle of young (~25 y) and older (~70 y) adults obtained 72 h after the final training session of a 12 week programme of either cycling HIIT (4 x 4 min at >90% peak oxygen uptake $\text{VO}_{2\text{peak}}$), RET (whole-body, 2-4 sets x 8-12 reps) or combined exercise training (HIIT and RET). They reported increased expression of 22 genes in older adults following HIIT, including the genes collagen type XIV alpha 1 chain (*COL14A1*) and lumican (*LUM*), with roles in extracellular matrix (ECM) organisation, and integrin subunit beta 2 (*ITGB2*), which is involved in integrin signalling. Increased basal expression of these genes may support enhanced ECM tensile strength, cell-to-ECM adhesions and mechanotransduction signalling (Goody *et al.*, 2015). Increased *COL14A1* and *LUM* expression has been reported following 12 weeks of ENT, suggestive of enhanced mechanotransduction to ECM components (Hjorth *et al.*, 2015), although the precise role of ECM reorganisation in facilitating muscle growth responses following aerobic exercise training remains unknown. HIIT also increased the expression of

11 genes in the older adults that were significantly downregulated prior to exercise training compared to younger adults including *MYLK4* (actin cytoskeleton regulation) and *KAZALDI* (insulin-like growth factor binding). Given both the *MYLK4* gene and protein expression have previously been shown to increase following SIT in younger adults (Miyamoto-Mikami *et al.*, 2018), increased mechanical support to the sarcomere to facilitate higher intensity contractions may be a characteristic molecular response to HIIT/SIT independent of age. Another key finding from the study by Robinson *et al.* (2017) was that the expression of genes upregulated with HIIT and resistance exercise training showed considerable overlap in both older (81 genes) and younger adults (88 genes). Collectively, results from these investigations (Miyamoto-Mikami *et al.*, 2018; Robinson *et al.*, 2017; Rundqvist *et al.*, 2019) provide evidence of transcriptional and translational responses implicated in muscle growth responses following exposure to different HIIT protocols of varying durations.

Following on from studies that have investigated transcriptional responses in skeletal muscle, two recent investigations have used deuterium oxide (D₂O) tracer methodology to measure MPS following HIIT. Scalzo *et al.* (2014) investigated the integrated *vastus lateralis* MPS response over the course of a 3 week SIT intervention (9 cycling sessions, 4-8 x 30 s, 100% VO_{2max}) in young (~23 y) adults. Contrary to their hypothesis and previous literature demonstrating no sex differences in exercise-induced MPS (Dreyer *et al.*, 2010; Short *et al.*, 2004), males had greater rates of both mixed (~0.40 vs. ~0.25%·day⁻¹) and cytoplasmic (~0.40 vs. ~0.29%·day⁻¹) protein synthesis compared to females. Regardless of the sex-based differences, post-intervention increases in mixed and cytosolic protein synthesis demonstrated that 3 weeks of SIT can stimulate increases in MPS in human skeletal muscle.

Bell *et al.* (2015) compared protein fractional synthetic rates (FSR) following a single session of either cycling sprints (10 x 1 min at ~95% HR_{peak}), resistance exercise (3 sets of leg press and leg extension at ~95% 10RM with last set to failure) or endurance exercise (30 min cycling at ~70% HR_{peak}) in untrained older (~67 y) men. Participants consumed D₂O for nine

days and muscle biopsies were obtained from the *vastus lateralis* on days 5-8 to estimate integrated myofibrillar and sarcoplasmic FSR during the 48-h period following each of the exercise bouts. Rates of myofibrillar FSR significantly increased in the 24 and 48 h period following the single bout of HIIT compared to rest (Bell *et al.*, 2015). The magnitude of the HIIT-induced increase in myofibrillar protein synthesis was less compared to resistance exercise (~50% versus ~80%) but greater than ENT (~10%) 24 h post exercise. Additionally, HIIT was the only exercise modality to increase sarcoplasmic protein synthesis 24 h post exercise (~25%), a response the authors suggested may be due to increased mitochondrial protein synthesis. The acute (Bell *et al.*, 2015) and short-term training-induced (Scalzo *et al.*, 2014) increases in MPS after HIIT demonstrate that HIIT can regulate the molecular machinery that underpins muscle hypertrophy. Whether acute rises in MPS align with HIIT-induced muscle hypertrophy is yet to be determined. Nevertheless, it is clear that skeletal muscle remodelling with HIIT may extend beyond established changes in oxidative capacity and substrate metabolism.

1.4.3 Effects of HIIT on Skeletal Muscle Fiber Cross-Sectional Area

Several studies have used histology-based techniques to assess whether short-term or prolonged HIIT can induce skeletal muscle fiber hypertrophy. Joannis *et al.* (2013) examined skeletal muscle fiber size following short-term HIIT using a 10 x 1 min cycling protocol performed three times per week over six weeks in untrained young women. The post-exercise training biopsy revealed that HIIT failed to increase muscle fiber size. In another investigation comprising two independent six-week study protocols, Joannis *et al.* (2015) implemented divergent aerobic-based training protocols to examine their effects on muscle fiber size. The first six-week study compared Tabata-style interval training to ENT performed three times per week by recreationally active young adults. In the second six-week intervention, young overweight adults performed three all-out 20 s cycling sprints against a resistance set to 0.05

kg·kg BM⁻¹ (with two minutes of active rest between work bouts) three times per week (Joanisse *et al.*, 2015). In agreement with their previous findings (Joanisse *et al.*, 2013), no changes in muscle fiber size with either aerobic-based exercise training intervention were observed (Joanisse *et al.*, 2015). Similar findings have been reported after eight weeks of either HIIT cycling (de Souza *et al.*, 2013) or running (Thorstensson *et al.*, 1975) in young men. Taken together, results from these studies (de Souza *et al.*, 2013; Joanisse *et al.*, 2013; Joanisse *et al.*, 2015; Thorstensson *et al.*, 1975) demonstrate that different short-term interval training protocols do not increase muscle fiber size in young adults. Whether middle-aged adults are more sensitive to potential anabolic effects of HIIT as demonstrated by increases in muscle fiber size is unknown.

1.4.4 Effects of HIIT on Body Composition and Whole-Muscle Morphology

The majority of studies that have reported changes in body composition following HIIT or SIT have utilised DEXA techniques whereby changes in total, appendicular lean tissue or fat-free mass are used as a proxy for skeletal muscle mass. A recent meta-analysis of 47 studies found no differences in body composition (i.e., increased lean body mass and/or decreased fat mass) between low volume HIIT and ENT or a non-exercise control (Sultana *et al.*, 2019). However, several other investigations that were not included in that meta-analysis because they did not meet inclusion criteria (e.g., no ENT group or non-exercising control), have detected changes in lean/fat-free mass in response to various HIIT protocols involving cycling (Bagley *et al.*, 2016; Blue *et al.*, 2017; Bruseghini *et al.*, 2015; Cassidy *et al.*, 2016; Dohlmann *et al.*, 2018; Gahreman *et al.*, 2016; Gillen *et al.*, 2013; Heydari *et al.*, 2012; Kong *et al.*, 2016; Maillard *et al.*, 2016; Robinson *et al.*, 2017; Sawyer *et al.*, 2016; Sculthorpe *et al.*, 2017; Sogaard *et al.*, 2017; Trapp *et al.*, 2008; Ziemann *et al.*, 2011), running (Ravnholt *et al.*, 2018; Stensvold *et al.*, 2010), rowing (Brown *et al.*, 2018), whole-body (Hwang *et al.*, 2016; Osawa *et al.*, 2014) and elliptical-based HIIT/SIT (Fex *et al.*, 2015). While some of these investigations

failed to detect changes in lean/fat free mass (Blue *et al.*, 2017; Bruseghini *et al.*, 2015; Cassidy *et al.*, 2016; Dohlmann *et al.*, 2018; Kong *et al.*, 2016; Sawyer *et al.*, 2016; Sogaard *et al.*, 2017; Stensvold *et al.*, 2010; Trapp *et al.*, 2008; Ziemann *et al.*, 2011), others reported an increase in lean/fat-free mass in response to HIIT (Bagley *et al.*, 2016; Brown *et al.*, 2018; Gahreman *et al.*, 2016; Gillen *et al.*, 2013; Heydari *et al.*, 2012; Macpherson *et al.*, 2011; Matsuo *et al.*, 2014a; Nybo *et al.*, 2010; Ravnholt *et al.*, 2018; Robinson *et al.*, 2017). Most studies that have observed increases in lean/fat-free mass with HIIT have incorporated training durations of ≥ 12 weeks undertaken by young (Brown *et al.*, 2018; Gahreman *et al.*, 2016; Heydari *et al.*, 2012; Robinson *et al.*, 2017), middle-aged (Bagley *et al.*, 2016; Nybo *et al.*, 2010) and older adults (Robinson *et al.*, 2017). Additionally, short-term HIIT interventions (6-8 weeks) have also induced increases in lean/fat-free mass in young (Gillen *et al.*, 2013; Macpherson *et al.*, 2011; Matsuo *et al.*, 2014a) and middle-aged adults (Ravnholt *et al.*, 2018).

As previously noted, these studies have estimated alterations in lean/fat-free mass using DEXA methodology where several limitations should be taken into account when considering exercise training-induced changes in body composition. For example, the precision (trueness) of whole-body lean mass measurements, as estimated from the coefficient of variation (CV) range from ~ 0.5 -1% depending on the densitometer used (Toombs *et al.*, 2012). Additionally, DEXA cannot distinguish muscle from intramuscular fluid and is affected by hydration status (Haun *et al.*, 2019b; Nana *et al.*, 2015). These factors have raised questions regarding the validity of DEXA-derived changes in muscle mass (Tavoian *et al.*, 2019). In contrast, techniques such as magnetic resonance imaging (MRI) and computed tomography (CT) are considered as reference methods for measuring whole-body and regional skeletal muscle mass (Mitsiopoulos *et al.*, 1998). However, notable limitations of MRI and CT include the cost and expertise to operate and maintain the scanners and in the case of CT, exposure to larger doses of ionising radiation. Nonetheless, available literature suggests HIIT can induce significant increases in lean mass in most adult age ranges.

1.4.5 Effects of HIIT on Muscle Strength

Little attention has been directed to the potential muscle strengthening effects of aerobic-based HIIT. Several studies have reported the effects of combined RET and HIIT on muscle strength (Del Vecchio *et al.*, 2019; Fyfe *et al.*, 2016; Spiliopoulou *et al.*, 2019), but investigations of the mode-specific effects of short-term HIIT on muscle strength are limited. Thorstensson *et al.* (1975) examined maximal isometric leg muscle strength following eight weeks of treadmill-based sprints ($\sim 20\text{-}40 \times 5 \text{ s}$ at $\sim 20 \text{ km}\cdot\text{h}^{-1}$ with $\sim 30 \text{ s}$ rest intervals) performed 3-4 days per week in a small cohort ($n=4$) of recreationally active young men. The running-based sprint protocol increased leg muscle strength. In contrast, de Souza *et al.* (2014) reported no change in leg press 1RM muscle strength following eight weeks of cycling HIIT ($\sim 15\text{-}20 \times 60 \text{ s}$ at $80\text{-}100\% W_{\text{max}}$ with $\sim 60 \text{ s}$ rest intervals) in previously untrained young men. Using a rowing HIIT intervention ($6 \times 60 \text{ s}$ ‘all-out’ with 3 min rest intervals), Buckley *et al.* (2015) reported no change in squat, bench press or deadlift 1RM muscle strength after six weeks of training in recreationally active young women. The results from these studies (Buckley *et al.*, 2015; de Souza *et al.*, 2014; Thorstensson *et al.*, 1975) suggest that exercise mode (e.g., running vs cycling/rowing) may play a role when increases in muscle strength after short-term HIIT are the goal. Given the small number of studies in young adults and differences in muscle strength assessments (e.g., maximal voluntary contraction vs 1RM testing) it is difficult to conclude whether short-term HIIT increases muscle strength, particularly in other age groups.

1.4.6 HIIT and Increased Protein Availability

One of the first studies to investigate the potential for protein ingestion to augment anabolic responses after HIIT was that of Coffey *et al.* (2011) in young ($\sim 21 \text{ y}$) recreationally active men. This work demonstrated post-exercise rates of myofibrillar protein synthesis were $\sim 48\%$ higher (~ 0.083 vs $\sim 0.056 \text{ \%}\cdot\text{h}^{-1}$) following repeated cycling sprints ($10 \times \text{six s}$, $0.75 \text{ N torque kg}^{-1}$ interspersed by 60 s of recovery) with ingestion of a pre-exercise meal containing

24 g of whey protein (4.8 g leucine) and 50 g maltodextrin or a placebo (Coffey *et al.*, 2011) (**Figure 1.6**). While protein ingestion and repeated sprints resulted in higher rates of myofibrillar protein synthesis, there was little effect of feeding on the rates of mitochondrial protein synthesis during ~4 h post-exercise recovery for nutrient or placebo treatments. The increase in myofibrillar protein synthesis with protein ingestion was concomitant with significant increases in phosphorylation status of Akt, mTOR, p70S6K and ribosomal protein S6 (rpS6).

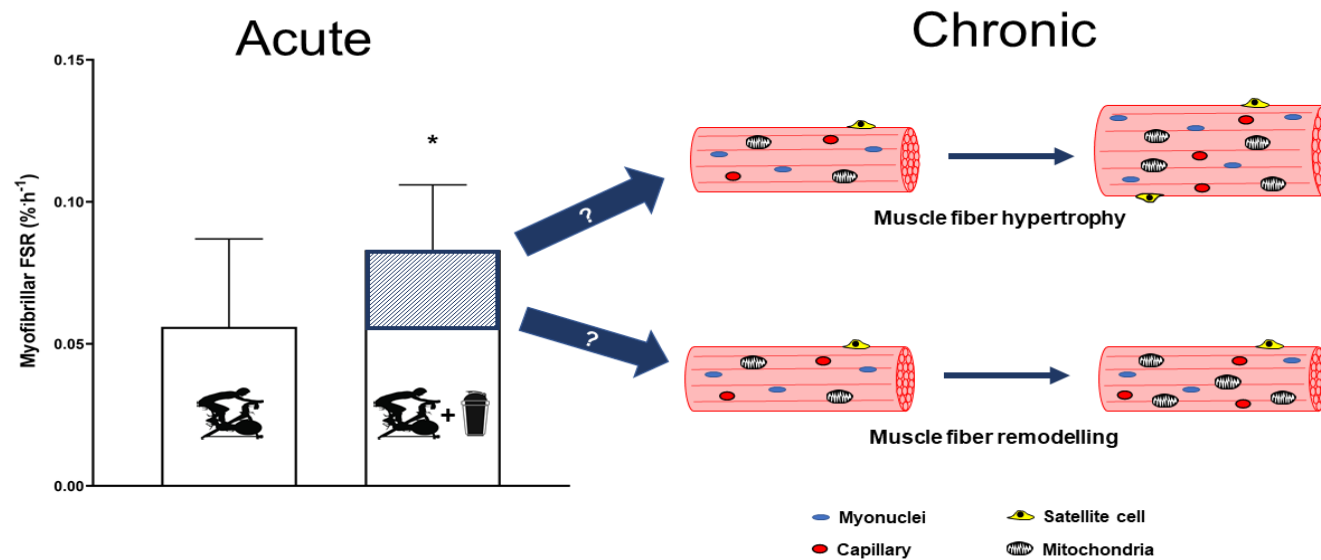


Figure 1.6 Acute rates of myofibrillar protein synthesis following cycling sprints with and without increased protein availability.

Myofibrillar fractional synthesis rate figure (left) redrawn from Coffey *et al.* (2011), with permission. Combined exercise and diet interactions that stimulate myofibrillar protein synthesis induce muscle hypertrophy when repeated over time (i.e., weeks to months). Previous evidence of a single bout of sprint interval exercise with protein ingestion significantly increasing rates of myofibrillar protein synthesis compared to a placebo condition (*) raises the possibility that sustained increases may promote increases in muscle fiber hypertrophy. However, the extent to which acute increases in rates of muscle protein synthesis form the basis of chronic muscle hypertrophy responses is equivocal with accumulating evidence in resistance exercise training models indicating such increases, in part, likely contribute to extensive muscle repair and remodelling of damaged proteins prior to facilitating muscle fiber hypertrophy (Damas *et al.*, 2016; Mitchell *et al.*, 2015).

Recently, Rundqvist *et al.* (2017) investigated the effect of an acute bout of SIT (3 x 30 s sprints separated by a 20 min recovery) in young (~26 y) men with the co-ingestion of 300 mg·kg BM⁻¹ of essential amino acids and 1 g·kg BM⁻¹ of maltodextrin 5 min before the first sprint and 15 min after each of the remaining sprints. Compared to a placebo treatment, cycling sprints in the fed state resulted in greater gene and protein expression of sodium-coupled neutral amino acid transporter 2 (SNAT2), Akt and mTORC1 (Rundqvist *et al.*, 2017). Moreover, post-exercise muscle FSR rates were higher (~15%) with the nutrient compared to placebo condition (Rundqvist *et al.*, 2017). The results from these studies provide evidence of acute changes to molecular networks that support myofibrillar (Coffey *et al.*, 2011) and mixed muscle (Rundqvist *et al.*, 2017) protein synthesis with high intensity interval-based exercise performed in the fed state, supporting the notion that HIIT undertaken with increased protein availability may be able to promote synergistic increases in muscle hypertrophy. Whether the cumulative effect of repeated HIIT sessions with increased protein availability can elicit similar or greater increases in rates of MPS over weeks/months remains an area for future investigation.

There is a paucity of information regarding the capacity for HIIT and increased protein availability to induce positive changes in muscle fiber hypertrophy or other measurements indicative of increases in skeletal muscle size/mass (e.g., MRI-derived muscle CSA/volume, DEXA-derived lean body mass, ultrasound-derived muscle thickness). Leuchtmann *et al.* (2020) observed no change in muscle fiber size following 12 weeks of cycling HIIT combined with post-exercise whey protein ingestion (30 g) in older men. However, total daily protein intake was not reported in that study. This is an important consideration as meeting a minimum daily dietary protein intake appears to be critical for exercise training-induced increases in muscle hypertrophy compared to protein feedings in close temporal proximity to an exercise bout (Morton *et al.*, 2017). In middle-aged adults with type 2 diabetes mellitus, 10 weeks of mixed model interval training (MMIT; consisting of HIIT and low-intensity high-volume resistance exercise performed on alternative days) combined with 20 g of whey protein before

and after each exercise session provided no further increases in *vastus lateralis* CSA compared a non-protein isoenergetic control beverage (Gaffney *et al.*, 2018). In that study, participants were encouraged to maintain dietary habits during the experimental period, although macronutrient intake was not reported (Gaffney *et al.*, 2018). As such it is difficult to determine if both total daily protein intake and distribution were adequate to support skeletal muscle protein accretion. Furthermore, given that an increase in $\text{VO}_{2\text{max}}$ but not 1RM was reported in that study (Gaffney *et al.*, 2018), it cannot be ruled out that the MMIT protocol may have contributed to blunted anabolic effects, particularly considering that combined strength and aerobic-based training typically negates some of the gains in muscle strength attained after single-mode exercise training (Hickson, 1980).

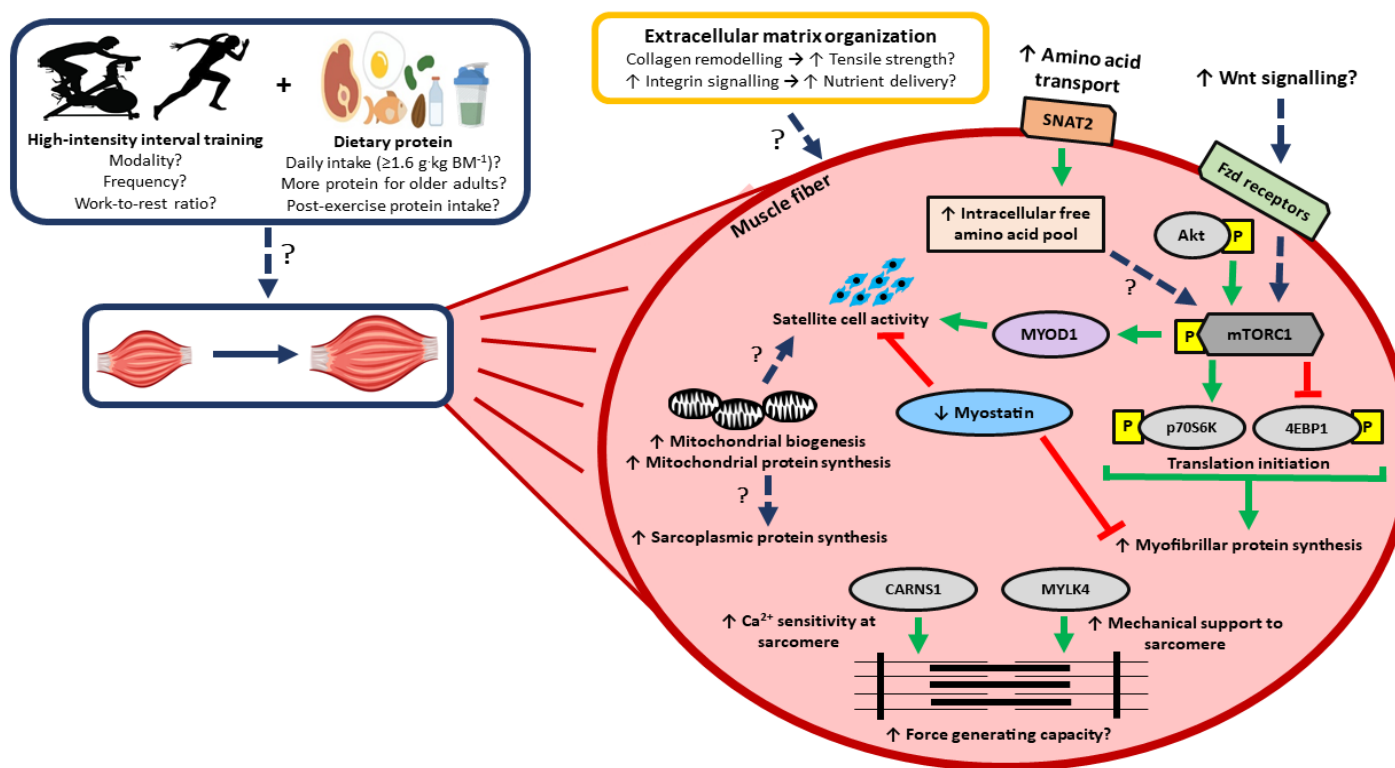


Figure 1.7 Schematic of putative factors that can be manipulated to induce muscle anabolism with combined HIIT and increased protein availability and cellular mechanisms that may underpin eventual gains in muscle mass.

Exercise modalities that increase total musculature under load during work periods (e.g., running, whole-body HIIT) and consuming a daily protein intake ($\geq 1.6 \text{ g} \cdot \text{kg} \text{ BM}^{-1}$) known to augment resistance exercise training-induced muscle hypertrophy (Morton *et al.*, 2017) are pivotal to uncovering the anabolic potential of HIIT and increased protein availability. At the myocellular level, HIIT and increased protein availability increases phosphorylation

of the Akt/mTOR pathway stimulating myofibrillar protein synthesis (Coffey *et al.*, 2011; Rundqvist *et al.*, 2017). HIIT increases sarcoplasmic (Bell *et al.*, 2015) and mitochondrial (Scalzo *et al.*, 2014) protein synthesis that over time may contribute to modest increases in muscle mass (Robinson *et al.*, 2017). Increased expression of the myogenic regulatory factor MYOD1, amino acid transporter SNAT2 and Wnt signalling transmembrane receptor FZD7 as well as decreased expression of negative muscle growth regulator MTSN represent some of the gene-encoding proteins that may contribute to pathways regulating muscle fiber size with HIIT and increased protein availability (Rundqvist *et al.*, 2017; Rundqvist *et al.*, 2019). Furthermore, increased protein expression of CARNS1 and MYLK4 may increase calcium handling to support higher intensity muscle contractions at the sarcomere (Miyamoto-Mikami *et al.*, 2018). Extracellular matrix remodelling may also play a supporting role in facilitating higher intensity muscle contractions by reorganization of collagen fibers to enhance transmission of tensile forces (Robinson *et al.*, 2017). 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; Akt, protein kinase B; BM, body mass; Ca²⁺, calcium; CARNS1, carnosine synthase; FZD7, frizzled class receptor 7; MYLK4, myosin light chain kinase 4; MYOD1, myoblast determination protein 1; MSTN, myostatin; mTOR, mammalian target of rapamycin; p70S6K, ribosomal protein S6 kinase beta-1; SNAT2, sodium-coupled neutral amino acid transporter 2. Green arrow denotes positive regulator of muscle growth, red arrow denotes negative regulator of muscle growth; blue dotted line denotes pathway that may be implicated in muscle growth with HIIT.

1.5 EXERCISE TRAINING CESSATION & SKELETAL MUSCLE ADAPTATION

1.5.1 Introduction to Exercise Training Cessation & Skeletal Muscle Mass Regulation

Another gap in our understanding of exercise-induced skeletal muscle adaptation responses are the effects of short-term exercise training cessation (i.e., detraining) subsequent to different exercise training modalities. While there are various models of ‘unloading’ in human skeletal muscle (**Figure 1.8**), detraining is the partial or complete loss of exercise training-induced adaptations due to a reduction or cessation in exercise frequency, intensity or duration (Mujika & Padilla, 2000). Similar to research examining the effects of muscle hypertrophy following exercise training, the effects of exercise training cessation in regard to skeletal muscle anabolism/catabolism have largely focused on prolonged periods of detraining (i.e., several months), typically in well-trained populations (e.g., athletes) (Andersen & Aagaard, 2010). Comparatively, less is known regarding changes to muscle size following short-term detraining in previously untrained populations.

It is important to define skeletal muscle responses resulting from the short-term cessation of exercise in non-athletic populations given that such individuals are also likely to experience interruptions to their exercise training routine (e.g., injury, illness, planned surgery, work-related travel preventing exercise) leading to skeletal muscle deconditioning. Indeed, even mild disuse resulting from two weeks of step reduction can decrease skeletal muscle mass in young (Krogh-Madsen *et al.*, 2010) and older adults (Breen *et al.*, 2013), and impair glycaemic control (Mcglory *et al.*, 2017b). RET and protein supplementation have been proposed as strategies to attenuate some of the catabolic events typically observed during periods of reduced physical activity (Oikawa *et al.*, 2019). Yet, exercise may not always be possible during periods of short-term inactivity (e.g., recovery from surgery or illness). As such, it is important to know how long exercise training-induced increases in skeletal muscle mass are retained following short-term periods of inactivity or detraining. However, studies addressing the exercise modality that best preserves anabolic short-term exercise training-

induced skeletal muscle adaptation responses following short-term exercise training cessation are few. Moreover, information regarding the molecular underpinnings of short-term detraining responses in untrained populations is scarce. In this thesis, detraining (**Figure 1.8**) was used to examine short-term exercise training cessation responses.



Figure 1.8 Common models used in human studies to determine the effects of unloading on skeletal muscle morphology.

1.5.2 Molecular Responses Implicated in Skeletal Muscle Anabolism and Catabolism

Following Exercise Training Cessation in Skeletal Muscle

In recent decades considerable scientific efforts have been made to identify cellular transducers involved in regulation of skeletal muscle mass during ‘catabolic’ scenarios resulting from skeletal muscle unloading (Vainshtein & Sandri, 2020). However, few studies have examined changes in the expression of many of the proteins in these pathways in human models of short-term exercise training and detraining. Such models are imperative for understanding the physiological relevance of exercise as a countermeasure to environmental conditions (e.g., detraining, physical inactivity) that ultimately decrease rates of muscle protein synthesis (Ferrando *et al.*, 1997; Mcglory *et al.*, 2017b) resulting in muscle atrophy.

Leger *et al.* (2006) examined anabolic and catabolic cell signalling gene and protein phosphorylation expression in recreationally active young men at rest before and after eight weeks of RET (post-exercise biopsy obtained ~48-72 h after final exercise session) and under

resting conditions following an eight week period of detraining (i.e., no exercise training). Following detraining, exercise training-induced increases in Akt^{Ser473} had returned to baseline levels although mTOR^{Ser2448} remained elevated, potentially indicating Akt-independent stimulation of mTOR (Memmott & Dennis, 2009). Interestingly, MuRF-1 and atrogin-1 mRNA expression increased following RET, a response the authors speculated may have been due to an increased demand to support a new level of muscle protein turnover. However, both MuRF-1 and atrogin-1 mRNA decreased to baseline after detraining. As it would be reasonable to expect a decrease in muscle protein turnover following eight weeks of detraining (Breen *et al.*, 2013; Mcglory *et al.*, 2017b), decreased MuRF-1 and atrogin-1 mRNA may reflect a return to basal gene expression.

Fritzen *et al.* (2020) assessed the protein expression of muscle regulatory factors MyoD and myogenin after six weeks of RET and subsequently every fortnight during eight weeks of detraining in untrained young and older adults. There was no change in MyoD or myogenin protein abundance after RET or detraining, irrespective of age (Fritzen *et al.*, 2020). There is a clear gap in knowledge regarding changes to molecular transducers that are linked to processes governing purported reductions in muscle mass in response to ceasing exercise training in human skeletal muscle. Elucidating these responses will provide further insight into the mode-specific molecular mechanisms modulating skeletal muscle mass.

1.5.3 Effects of Short-Term Exercise Training Cessation on Skeletal Muscle Fiber Cross-Sectional Area Following Exercise Training

Several studies that have investigated the effects of short-term exercise training cessation on muscle fiber CSA were conducted in well-trained populations who stopped their habitual training regime. In endurance-trained athletes, there was no decrease in skeletal muscle fiber size after 2-3 weeks of exercise training cessation (Coyle *et al.*, 1984; Houmard *et al.*, 1992). In contrast, two weeks of detraining was sufficient to decrease type II muscle fiber size

in strength/power-based athletes (Hortobágyi *et al.*, 1993; Houmard *et al.*, 1993). The effects of short-term exercise training cessation on skeletal muscle fiber size has also been assessed in non-athletic populations. Kadi *et al.* (2004) assessed skeletal muscle fiber size in untrained young men at different time points during training cessation following 12 weeks of RET. The RET-induced increase skeletal muscle fiber size was retained after day 10, but not day 30, of detraining. Klausen *et al.* (1981) reported similar findings following eight weeks of cycling ENT in untrained young adults. In that study, type I, type IIa and type IIb skeletal muscle fiber size decreased after four weeks of detraining (Klausen *et al.*, 1981). More recently, Fritzen *et al.* (2019) investigated the effects of short-term ENT and subsequent detraining using a one-legged knee extensor model of continuous (40 min at 70% $\text{VO}_{2\text{peak}}$) and interval-style exercise (5 x 5 min at 95% $\text{VO}_{2\text{peak}}$) in untrained young men. There was no change in type I or II muscle fiber size after exercise training or detraining in the experimental leg. Taken together, results from prolonged RET and short-term ENT interventions in previously untrained young adults suggest that decreases in skeletal muscle fiber size are detected after ~2-4 weeks of detraining. Whether skeletal muscle atrophy occurs in a similar timescale in middle-aged adults as well as following short-term HIIT has not been investigated.

1.5.4 Effects of Short-Term Exercise Training Cessation on Body Composition and Whole Muscle Morphology Following Exercise Training

Measurements of lean mass and muscle morphology are important markers of changes in skeletal muscle mass during periods of reduced physical activity. With regard to short-term exercise training and detraining, such time frames represent the perioperative time window (i.e., the immediate weeks prior to and following surgery) faced by many clinical populations (e.g., planned elective surgery) (McQueen *et al.*, 2015). Given short-term pre-operative exercise training has the capacity to attenuate lean mass losses (Tew *et al.*, 2018), it is important to determine which exercise modes protect against potential reductions in skeletal muscle mass

during the post-operative period. However, there is a paucity of information concerning short-term exercise training and detraining in healthy adults who more closely represent clinical populations (e.g., age, training status). As such, further work into how middle-aged adults, and even older adults, respond to stopping short-term exercise training with regard to lean mass may aid clinicians tasked with peri-operative decisions regarding exercise prescription.

Pre-operative exercise training has been studied in various clinical settings in individuals who are susceptible to muscle loss (Gillis *et al.*, 2018; Gustafsson *et al.*, 2019; Jones *et al.*, 2017; Ratnayake *et al.*, 2018). Due to the high heterogeneity of patient populations, generalised ‘optimal’ pre-operative exercise programming is unclear (Tew *et al.*, 2018). Given exercise prescription principles are in essence the same for healthy and clinical populations (Spruit *et al.*, 2013), findings in healthy middle-aged adults may inform clinical exercise prescriptions. Indeed, one contemporary issue surrounding pre-operative exercise training prescription, particularly in the short-term, is whether concurrent resistance and endurance exercise training, as usually prescribed (Tew *et al.*, 2018), is most effective for maximising post-operative outcomes (Topal *et al.*, 2019). Tailoring exercise training programmes to suit the patient’s needs is important, but limited information exists surrounding lean mass responses to short-term single-mode exercise training. Thus, characterising single-mode exercise training responses in adults may provide a basis to help guide skilled clinicians by adapting the movement (e.g., mode, intensity) but aiming to achieve similar outcomes (e.g., increased muscle mass).

In response to short-term detraining (~2-4 weeks), lean mass remains elevated above baseline levels following prior short-term (eight weeks) (Brandner *et al.*, 2019) but not prolonged (24 weeks) RET (Spence *et al.*, 2011) in untrained young men. In contrast, Lovell *et al.* (2010b) observed no decline in lean mass after four weeks of detraining following 16 weeks of RET in untrained older men. Regarding changes in muscle morphology, 6-8 weeks of RET was sufficient to prevent declines in *quadriceps femoris* muscle thickness (Brandner *et al.*,

2019), *vastus lateralis* CSA (ultrasound) (McMahon *et al.*, 2014), *triceps brachii* and *pectoralis major* CSA (MRI) (Ogasawara *et al.*, 2011) after ~2-4 weeks of detraining in young adults. Conversely, (Brandner *et al.*, 2019) observed a decrease in *biceps brachii* muscle thickness below post-RET levels after four weeks of detraining.

Fewer studies have investigated short-term detraining lean mass and muscle morphology responses after ENT. Spence *et al.* (2011) reported that lean mass remained elevated compared to baseline after six weeks of detraining that followed six months of ENT in untrained young men, while Lovell *et al.* (2010a) reported that leg lean mass returned to baseline values after four weeks of detraining following 16 weeks of ENT in older men. Taken together, RET and ENT are capable of maintaining lean mass and various measures of whole-muscle size. Results from these studies, however, have largely been conducted in young adults. As such, further work into middle-aged adults is needed to characterise skeletal muscle adaptation to short-term exercise training cessation who remain underrepresented in the literature. Moreover, little research has focused on HIIT and detraining-induced whole-muscle mass/size responses.

1.5.5 Effects of Short-Term Exercise Training Cessation on Muscle Strength Following Exercise Training

Muscle strength is a critical determinant of physical function, particularly with advancing age (Buchner & de Lateur, 1991). Three to four weeks of detraining does not attenuate prolonged RET-induced increases in maximal lower body 1RM muscle strength in young (Spence *et al.*, 2011) and middle-aged (Häkkinen *et al.*, 2000) and (Lovell *et al.*, 2010b). Similarly, short-term RET followed by short-term periods of detraining can maintain lower training-induced muscle strength gains in untrained young adults (Brandner *et al.*, 2019). Regarding upper body muscle strength, four (Brandner *et al.*, 2019) but not three (Ogasawara *et al.*, 2011), weeks of detraining induced declines in short-term RET-induced bench press 1RM

muscle strength. Notably, in the study by Ogasawara *et al.* (2011), participants only performed upper body resistive exercise (i.e., bench press) throughout the entire RET which may, in part, explain why strength gains were retained after detraining.

In response to prolonged ENT, increases in squat 1RM muscle strength are retained after short-term detraining in young (Spence *et al.*, 2011) but not older men (Lovell *et al.*, 2010a). Similarly, bench press 1RM muscle strength is also retained after prolonged RET and subsequent short-term detraining in young men (Spence *et al.*, 2011). Similar to other exercise training-detraining models, the effects of HIIT-induced adaptation responses remain largely understudied with regard to exercise training cessation in all age groups. Additionally, no study has compared exercise training modality following short-term detraining.

1.6 OVERALL AIMS OF THE THESIS

Appropriate exercise training regimens when undertaken in combination with optimal nutritional availability are the most successful primary lifestyle interventions to attenuate loss of skeletal muscle with advancing age. However, studies assessing anabolic skeletal muscle adaptive responses in middle-aged adults remain underrepresented in exercise science literature. Time constraints may place limitations on the types of exercise middle-aged adults choose to regularly perform for general health, including retention of skeletal muscle mass. As such, ‘time-efficient’ options may be appealing for such individuals.

Skeletal muscle adaptive responses to exercise training are mode dependent. Indeed, prolonged RET is known to increase skeletal muscle fiber size, mass and strength. On the other hand, short-term aerobic-style exercise training (i.e., ENT and HIIT) primarily increases mitochondrial density and whole-body markers of cardiorespiratory fitness. Results from studies examining the ‘anabolic’ effects of aerobic exercise training reveal the potential for modalities traditionally associated with improvements in endurance capacity (e.g., cycling, running) to also stimulate select genes and proteins that play important regulatory roles in

muscle hypertrophy and strength gains. Higher intensity aerobic-based exercise may be a practical option to achieve anabolic skeletal muscle adaptations thereby better ‘replicating’ RET than ENT. Yet, limited investigations have explored the short-term anabolic effects of HIIT. Moreover, no previous studies have directly compared HIIT, RET and ENT within the same study design. Accordingly, the primary aim of this thesis was to investigate the effects of six weeks of HIIT, combined with increased dietary protein availability, on skeletal muscle gene and protein expression, skeletal muscle fiber CSA, lean mass, muscle thickness and maximal muscle strength. A secondary aim was to compare these responses to RET and ENT (Figure 1.9).

While the literature is replete with exercise responses, the effects of exercise training cessation are less clear. As such, the third aim of this thesis was to investigate the effects of a short-term ‘detraining’ period (2.5 weeks) that proceeded exercise training to define whether skeletal muscle adaptive responses are maintained over a timeframe replicating brief interruptions to exercise (e.g., injury, illness).

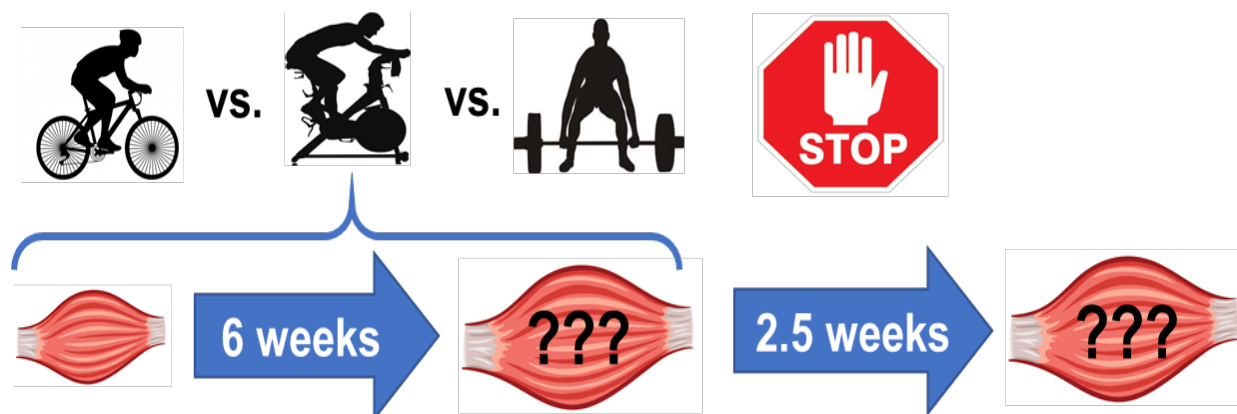


Figure 1.9 Simplified schematic of thesis project design.

LINKING CHAPTERS 1 & 2

Chapter 1 provided an overview of anabolic responses, from cellular to whole-body, following short-term exercise training. In particular, the review highlighted the paucity of knowledge regarding short-term exercise training, particularly after HIIT, and muscle fiber size responses. Chapter 1 emphasised a need for more investigations to be undertaken in middle-aged adults, who are currently understudied and who represent an important cohort who could, potentially, attenuate some of the deleterious effects of muscle loss later in life. Another gap identified in the review was the lack of information regarding the efficacy for increased protein availability to augment skeletal muscle adaptations during short-term exercise training, particularly in response to ENT and HIIT. Finally, limited information exists regarding skeletal muscle mass, size and expression of genes and proteins regulating muscle morphology after short-term periods of detraining, representative of brief interruptions to exercise (e.g., injury illness) routines among adults, particularly following HIIT and ENT.

The investigation described in Chapter 2 partially fills one of these knowledge gaps and directly compares skeletal muscle fiber, whole muscle and whole-body responses between short-term RET, HIIT and ENT in sedentary, middle-aged adults. Furthermore, the work presented in Chapter 2 provides novel information as to how a period of short-term exercise training cessation from each of these exercise modalities directly impacts the spectrum of adaptation responses measured. Individuals typically halt exercise training for several reasons including illness, injury or simply due to a 'lack of time'. Thus, determination of whether one exercise mode (i.e., HIIT) might preserve adaptation responses to a greater magnitude than other modalities (i.e., endurance-based training) will advance current knowledge of the time course that beneficial effects of exercise training are maintained. The results of the study described in Chapter 2 provides practical application regarding the durations of exercise training and detraining periods mimicking the peri-operative period for certain clinical

populations (e.g., planned surgery). An attempt to broaden the potential scope of the findings of this study is proposed, namely that short-term single-mode exercise training, particularly HIIT, may be a mode of exercise that can concomitantly improve multiple facets of physical function in the face of strict exercise time constraints (e.g., prior to surgery).

CHAPTER 2

Experimental Chapter 1

Chapter 2 has been adapted from the following published original research article:

Callahan, M.J., Parr, E.B., Snijders, T., Conceição, M.S., Radford, B.E., Timmins, R.G., Devlin, B.L., Hawley, J.A., Camera, D.M. Skeletal muscle adaptive responses to different types of short-term exercise training and detraining in middle-aged men. *Medicine & Science in Sport & Exercise*. doi: 10.1249/MSS.00000000000002684.

2.1 ABSTRACT

Background: Whether short-term single-mode exercise training can improve physical fitness prior to reduced physical activity (e.g., post-surgery recovery) is not well characterised in clinical populations nor middle-aged adults. This study investigated skeletal muscle adaptive responses following endurance exercise training (ENT), high-intensity interval training (HIIT) or resistance exercise training (RET), and a subsequent period of detraining, in sedentary, middle-aged men.

Methods: Thirty-five sedentary, males (39 ± 3 y) were randomised to parallel groups for six weeks of either ENT ($n=12$), HIIT ($n=12$) or RET ($n=11$) followed by 2.5 weeks of detraining. Muscle fiber characteristics, body composition, muscle thickness, muscle strength, aerobic capacity, resting energy expenditure and glucose homeostasis were assessed at baseline, and after exercise training and detraining.

Results: Lean mass increased after RET and HIIT ($+3.2 \pm 1.6\%$ and $+1.6 \pm 2.1\%$, $P < 0.05$). Muscle strength (sum of leg press, leg extension and bench press 1RMs) increased after all exercise training interventions (RET: $+25 \pm 5\%$; HIIT: $+10 \pm 5\%$; ENT: $+7 \pm 7\%$, $P < 0.05$). Aerobic capacity increased only after HIIT and ENT ($+14 \pm 7\%$ and $+11 \pm 11\%$, $P < 0.05$). Type I and II muscle fiber size increased for all groups post-exercise training (main effect of time, $P < 0.05$). Following a period of detraining, the gains in lean mass and maximal muscle strength were maintained in RET and HIIT groups, but maximal aerobic capacity declined below post-exercise training levels in HIIT and ENT ($P < 0.05$).

Conclusion: Six weeks of HIIT can induce widespread exercise training adaptations prior to detraining in middle-aged men. Exercise training-induced increases in aerobic capacity declined during 2.5 weeks of detraining but gains in lean mass and muscle strength were maintained.

2.2 INTRODUCTION

Exercise training enhances physical fitness (muscle strength and aerobic capacity) resulting in marked improvements in metabolic health and functional capacity (Hawley *et al.*, 2014). As such, intense exercise training prior to a period of forced or planned inactivity (i.e., injury or post-surgery) has become common practice as a strategy to augment pre-operative physical fitness and post-operative recovery (Gustafsson *et al.*, 2019; Tabesh *et al.*, 2019; Topal *et al.*, 2019). Pre-operative exercise training prescription is based upon general exercise training guidelines emphasising combined endurance and resistance exercise training (Tew *et al.*, 2018). However, whether implementation of both exercise modalities reflects optimal programming for all pre-operative settings is questionable (Pouwels *et al.*, 2016; Scheede-Bergdahl *et al.*, 2019).

While combined exercise training can induce robust changes in muscle strength and aerobic capacity over extended time periods (≥ 12 weeks) (Carli & Zavorsky, 2005), many individuals have to undergo surgery at short notice, and do not have time to undertake such exercise training regimens (Topal *et al.*, 2019). As such, it is important to determine the short-term, mode-specific effects of exercise training on multiple components of physical fitness. Defining short-term muscle adaption responses to different exercise training modalities in healthy middle-aged adults is important to provide evidence-based guidelines for short-term pre-operative exercise training programming.

Six weeks of aerobic-based exercise training increases aerobic capacity (Burgomaster *et al.*, 2008; Dohlmann *et al.*, 2018; Higgins *et al.*, 2016; Sogaard *et al.*, 2017). However, the short-term effects of such training interventions on muscle strength are less clear. Another important aspect of physical fitness is skeletal muscle mass which is necessary for mobility as well as whole-body glycaemic control (Lee *et al.*, 2017b; Reid *et al.*, 2008). Individuals with low muscle mass before surgery are at increased risk of adverse post-operative outcomes such as major surgery-related complications, prolonged hospital length of stay, morbidity and

mortality (Jones *et al.*, 2017; Kazemi-Bajestani *et al.*, 2016; Moskven *et al.*, 2018; Ratnayake *et al.*, 2018). Yet, comparisons between short-term exercise training modalities on markers of whole-body and regional muscle mass in middle-aged adults are lacking.

Another knowledge gap regarding muscle adaptation responses are the effects of short-term exercise training cessation (i.e., detraining) subsequent to divergent exercise training modalities. Detraining is the partial or complete loss of exercise training-induced adaptations due to a reduction or cessation in exercise frequency, intensity or duration (Mujika & Padilla, 2000). In the early post-operative period (i.e., the first few weeks following surgery), exercise training may not be feasible due to pain, nausea or physical restrictions (Bowyer & Royse, 2016). As little as two weeks of reduced physical activity can induce catabolic events in skeletal muscle, resulting in decreased muscle mass (Breen *et al.*, 2013; Krogh-Madsen *et al.*, 2010) and impaired glycaemic control (Mcglory *et al.*, 2017b). Whether short-term exercise training adaptations are maintained after a short detraining period in middle-aged adults is unknown. Ultimately, clarification of short-term single-mode exercise training and detraining responses in healthy middle-aged adults will help to inform pre-operative exercise training programming, particularly for populations tasked with time constraints prior to surgery.

In the present study, the hypothesis that six weeks of either endurance, resistance, or high-intensity interval exercise training would induce divergent anabolic and metabolic skeletal muscle adaptive responses in middle-aged men was tested. Among the anabolic responses, *vastus lateralis* muscle fiber cross-sectional area (CSA) was assessed after exercise training and detraining. Here, it was hypothesised that high-intensity interval training (HIIT), due to its closer resemblance in contractile intensity/activity with resistance exercise training (RET), would induce a greater increase in muscle fiber CSA compared to endurance exercise training (ENT), although this increase would be less in magnitude compared to RET.

2.3 METHODS

2.3.1 Participants & ethics approval

Thirty-nine males (age 39 ± 3 years; body mass 94 ± 13 kg; body mass index [BMI] 29 ± 3 kg·m⁻²), who were not meeting current national physical activity guidelines (Brown *et al.*, 2012) for the six months prior to recruitment, volunteered to participate in this study. As four participants withdrew from the study, a total of 35 participants completed the protocol and were included for analysis (**Figure 2.1**). All participants completed the *Exercise & Sports Science Australia Adult Pre-exercise Screening Tool* (ESSA, 2017) to identify individuals who may be at a higher risk of an adverse event while exercising, in which case clearance to participate was sought from a medical practitioner prior to participation. Exclusion criteria included: age <35 or >45 y, BMI <25 or >35 kg·m⁻², smoking, type 2 diabetes mellitus, regular use of non-steroidal anti-inflammatory medication, scheduling conflicts prohibiting morning exercise session attendance and previous injuries exacerbated by exercise. Written informed consent was obtained from all participants included in the study. The study was approved by the Australian Catholic University Human Research Ethics Committee (#2017-104H), prospectively registered online (ACTRN12617000894392; 19/06/2017) and conducted in accordance with the most recent revisions of the Declaration of Helsinki. The study was undertaken at the exercise physiology laboratories at the Australian Catholic University's St Patrick's campus (Fitzroy, VIC, Australia).

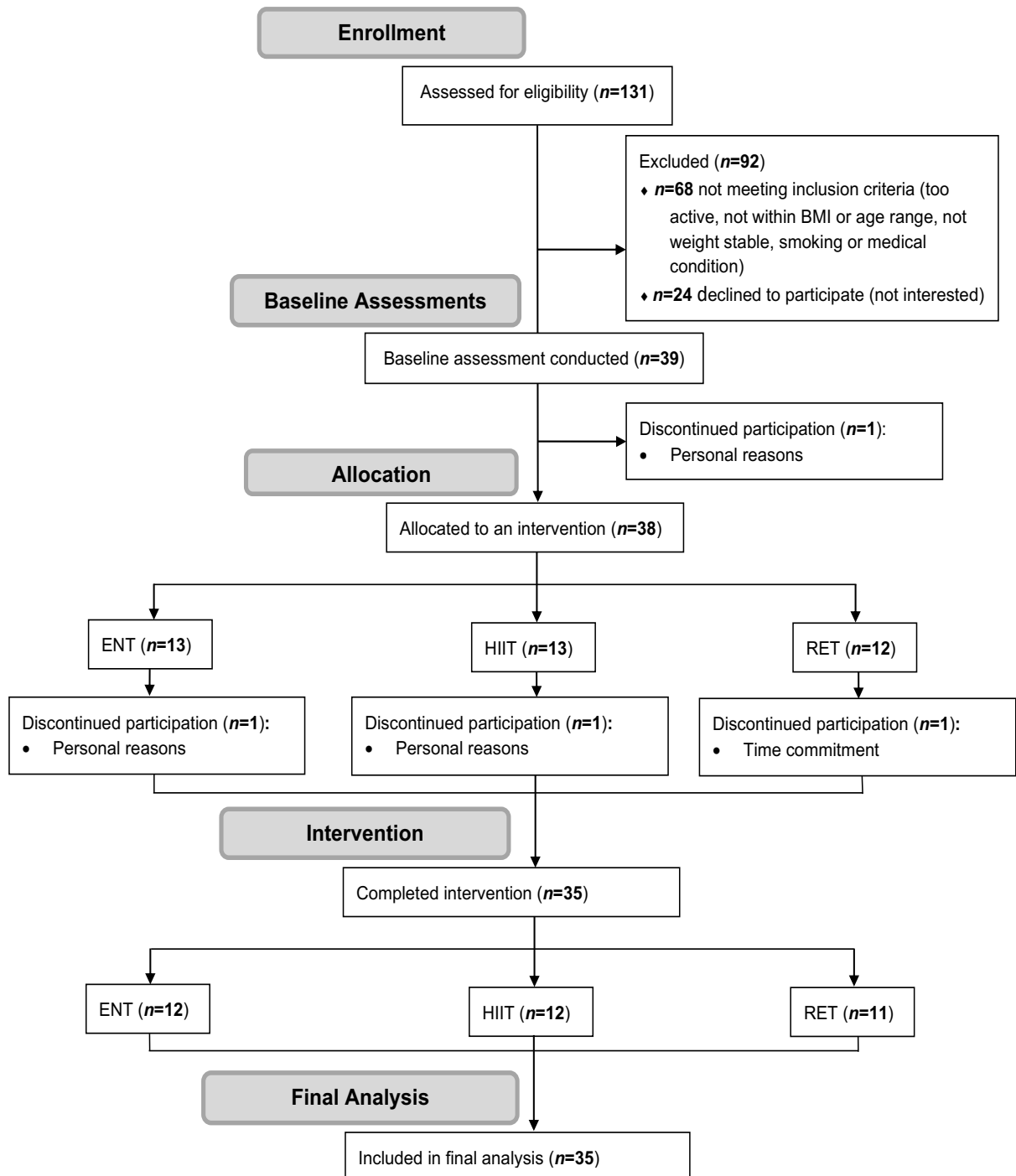


Figure 2.1 Participant recruitment flow.

2.3.2 Study design and overview

The study was conducted in a parallel groups design. Participants reported to the laboratory on ten occasions for study measures and a further 18 occasions for supervised exercise training sessions (**Figure 2.2**). During preliminary testing, participants presented to the lab on four occasions over ~two weeks. Visits for baseline measurements took place ~24-48 h apart in the following order: at visit one, body composition scan (DEXA), two-hour oral glucose tolerance test (OGTT) and initial diet consult; at visit two, a resting metabolic rate (RMR) test and resting *vastus lateralis* muscle biopsy; at visit three, a *vastus lateralis* two-dimensional (2D) B-mode ultrasound and maximal cycling exercise test ($\text{VO}_{2\text{peak}}$), and at visit four, a maximal strength test (1RM) and follow up diet consult. After completion of all preliminary testing, participants were allocated to one exercise training group:

During the six weeks of exercise training, participants performed three sessions per week on alternate days (e.g., Monday, Wednesday and Friday) of either cycling endurance exercise training (ENT), cycling high-intensity interval training (HIIT) or whole-body resistance exercise training (RET). After six weeks of exercise training, all measurements taken at baseline were repeated (Post). Thereafter, participants were instructed to refrain from exercise training and maintain their activities of daily living for 2.5 weeks after which all measurements were repeated for the final time (detraining; DT).

	Habitual diet		Study diet									
	Preliminary testing		Exercise training						Detraining			
	Study week											
	-2	-1	1	2	3	4	5	6	7	8	9	10
Daily diet recording	★	★	★	★	★	★	★	★	★	★	★	★
Face-to-face diet consult	🏠	🏠			🏠			🏠			🏠	
Muscle biopsy	↑							↑			↑	
Oral glucose tolerance test	+							+			+	
Body composition scan	●							●			●	
Resting energy expenditure test	◆							◆			◆	
Maximal oxygen uptake test		➡				➡			➡			➡
Maximal muscle strength tests		✖				✖			✖			✖
Muscle ultrasound		■							■			■
Physical activity monitoring	📶	📶				📶				📶	📶	📶

Figure 2.2 Study design.

Overview of when measurements were taken throughout the study. Exercise training intensities were adjusted where necessary at week 4. All measurements were obtained under resting conditions except for the post-exercise training muscle biopsy collected ~48 h following the final exercise training session (end of week 6). Post-exercise training testing (Post) took place across study weeks 6 and 7 and post-detraining testing (DT) across study weeks 9 and 10.

2.3.3 Exercise training protocols

All groups performed three morning exercise sessions per week for six weeks. Progressive overload was applied to all exercise training protocols. The primary aim of the HIIT and ENT protocols was to increase aerobic capacity while the overarching goal of the RET protocol was to increase skeletal muscle mass and strength. Participants in HIIT and ENT groups wore a heart rate monitor (Polar H2, Polar, Australia) during each exercise session and RPE was obtained at regular intervals (i.e., at the conclusion of a work period) using Borg's CR6-20 scale (Borg, 1982). At the beginning of week four, participants in the HIIT and ENT groups performed a VO_2peak test without breath analysis to reassess MAP. At the same time point, individuals in the RET group performed 1RM testing to reassess muscle strength. Based on results from week four exercise testing, training intensities were adjusted accordingly.

2.3.3.1 Endurance exercise training

A three-minute warm-up (100 W) preceded each training session on a stationary cycle ergometer. Total exercise session duration varied between 30-52 min comprised of 5-8 min work periods at 50-75% peak power output (PPO) with one minute of active rest at 50 W. A three-minute cool-down at 50 W followed the final rest period. The duration and intensity of work periods increased throughout the exercise training programme. Further details can be found in **Appendix A**.

2.3.3.2 High-intensity interval training

A three-minute warm-up (100 W) preceded each training session on a stationary cycle ergometer. Total exercise session duration varied between 13-23 min comprised of 30-60 s work periods at 90-130% PPO with one minute of recovery at 50 W. The number of repetitions varied from 8-15 depending on the work period duration for that session. A three-minute cool-

down at 50 W followed the final rest period. The duration and intensity of work periods increased throughout the exercise training programme. Further details can be found in **Appendix A**.

2.3.3.3 Resistance exercise training

The RET protocol included upper (bench press, seated dumbbell overhead press, incline dumbbell chest press, *latissimus dorsi* pulldown and pulley seated row) and lower (45° incline bilateral leg press, bilateral knee extension, dumbbell stationary lunge and dumbbell step ups) body weight-bearing exercise using pulley machines and free weights. A three-minute warm-up (50 W) on a cycle ergometer preceded each RET session. Sets ranged from 3-4 and repetitions from 9-12 at 60-80% 1RM. Three minutes of rest was standardised between sets for all exercise movements. A three-minute cool-down at 50 W on a cycle ergometer concluded each RET session. Further details can be found in **Appendix A**.

2.3.4 Maximal strength testing

Maximal upper and lower body muscle strength was assessed via a battery of one-repetition maximum (1RM) tests including bilateral leg press (45° incline), bilateral knee extension and bench press. A series of sets (3-5, 2-8 repetitions) at increasing submaximal weights were lifted until the participant reported a rating of perceived exertion (RPE) of ~16 using Borg's CR6-20 scale (Borg, 1982). Each 1RM attempt was followed by five minutes of rest. The 1RM for each exercise was determined in isolation (i.e., exercises were not alternated). A maximum of five 1RM attempts were allowed per exercise. The 1RM achieved for each exercise was used to determine RET intensities (%1RM). Further details can be found in **Appendix A**.

2.3.5 Peak aerobic capacity

Participants performed a progressive incremental cycling exercise test on a stationary ergometer (Lode, Excalibur sport, Groningen, Netherlands) to determine peak oxygen uptake ($\text{VO}_{2\text{peak}}$) and maximal aerobic power (MAP). Participants were fitted with a heart rate monitor and performed a five-minute warm-up at a power output of one watt per kilogram of body weight ($\text{W}\cdot\text{kg}^{-1}$) whilst wearing a mouthpiece (Hans Rudolf Inc., Kansas, USA) for collection of expired breath connected to a calibrated (O_2 : 16%, CO_2 : 4%) metabolic cart (TrueOne 2400, ParvoMedics, Utah, USA). During the test, power output increased by 25 W every 2.5 min (Hawley & Noakes, 1992). Within the final 15 sec of each stage, participants were asked for their rating of perceived exertion (RPE) using Borg's CR6-20 scale (Borg, 1982). Participants were required to maintain a cadence >70 rpm until volitional exhaustion. The MAP achieved at $\text{VO}_{2\text{peak}}$ was used to determine exercise training intensities (W) for ENT and HIIT.

2.3.6 Body composition

Participants reported to the laboratory in an overnight fasted state between 0630-0730 h where a dual-energy X-ray absorptiometry scan (DEXA; GE Lunar iDXA Pro, enCORE software Version 16, General Electric, Boston, MA, USA) was conducted (Nana *et al.*, 2015) to assess lean (total and regional) and fat mass (coefficient of variation (CV) of repeat measures on densitometer: $<1.5\%$).

2.3.7 Muscle thickness

The left and right *vastus lateralis* were scanned by two-dimensional (2D) B-mode ultrasound (frequency, 12 MHz; depth, 8 cm; field of view, 14 x 47 mm) (GE Healthcare Vivid-

/, Wauwatosa, WI) to determine muscle thickness from ultrasound images taken along the longitudinal axis of the muscle belly. Further details can be found in **Appendix A**.

2.3.8 Oral glucose tolerance test

A standard (75 g, 300 mL) two-hour oral glucose tolerance test (OGTT), with 30 min sampling, was conducted to exclude those participants who may have had type 2 diabetes mellitus. Fasted blood glucose was determined using a handheld glucometer (Accu-Chek Performa II, Roche Diagnostics Ltd., Basel, Switzerland). If blood glucose was $<6.9 \text{ mmol}\cdot\text{L}^{-1}$ (i.e., absence of type 2 diabetes), participants consumed a glucose solution containing 75 g of glucose (PoC Diagnostics, North Rocks, NSW, Australia). Blood glucose was again determined using the handheld glucometer immediately following collection of the final OGTT sample. Participation was confirmed if the sample concentration was $<11.0 \text{ mmol}\cdot\text{L}^{-1}$ (i.e., absence of glucose intolerance). Blood samples were centrifuged at 4°C for 10 min at 4,095 rpm and plasma aliquots were stored at -80°C until analysis.

2.3.9 Skeletal muscle biopsy

Participants were provided a standardised meal (45% carbohydrate, 25% protein and 30% fat; 33% of total daily energy intake ($\sim 3700\text{--}4300 \text{ kJ}$) based on the Cunningham equation (Cunningham, 1980) for consumption the evening prior to a muscle biopsy. Participants reported to the laboratory in an overnight fasted state ($\sim 10 \text{ h}$) between 0630-0730 h on the day of a muscle biopsy. A resting percutaneous muscle biopsy from the *vastus lateralis* ($\sim 100\text{--}200 \text{ mg}$) was obtained, under local anaesthesia (2–3 mL of 1% Xylocaine) using a Bergström needle (Bergstrom, 1975) modified for manual suction. One to two pieces of the muscle tissue sample ($\sim 30\text{--}40 \text{ mg}$ per piece) were mounted on a water-soluble compound (Tissue-Tek Optimal Cutting Temperature, Sakura Finetek, Netherlands), frozen in liquid nitrogen cooled

isopentane and stored at -80 °C until analysis. The remaining sample was also frozen in liquid nitrogen and stored at -80 °C until analysis. The post-exercise training muscle biopsy was obtained ~48 h following the final exercise session. The DT muscle biopsy was obtained at rest.

2.3.10 Resting energy expenditure

Participants reported to the laboratory in an overnight fasted state (~10 h) between 0630-0730 h and underwent measures to estimate resting energy expenditure (REE). A calibrated (O₂: 16%, CO₂: 1%) metabolic cart (TrueOne 2400 with dilution pump, ParvoMedics, Utah, USA) was used to capture expired oxygen and carbon dioxide. Participants lay supine on a bed in a dimly lit room for ~25 min. The first 10 min of expired gas was used to establish a steady-state with the remaining 15 min used for estimation of REE (kcal). In this study, the CV for REE measurements was 5.2%.

2.3.11 Physical activity

A physical activity monitor (activPAL3 tri-axial accelerometer, PAL-technologies Ltd., Glasgow, Scotland) was worn on the thigh to track habitual physical activity for all of the preliminary testing period, one week of exercise training (i.e., week four) and all of the detraining period. The physical activity monitor was changed weekly. ActivPAL-derived daily step count and proportions of time spent sitting, standing, stepping and cycling were estimated by exporting data files from the associated proprietary software (PAL Software Suite Version 8.10, PAL-technologies Ltd., Glasgow, Scotland).

2.3.12 Dietary intervention and analysis

For the both the exercise training and detraining periods, participants were prescribed a protein intake of ~ 1.4 (upper limit of 1.6) g·kg BW⁻¹·d⁻¹ deemed optimal for stimulating positive muscle protein turnover in exercising adults (Jager *et al.*, 2017), predominantly from foods already consumed as part of their habitual diet. A 40 g serve of whey protein powder supplement (Whey Protein Concentrate, Bulk Nutrients, Tasmania, Australia) providing ~ 30 g of protein was consumed by all participants immediately following exercise to optimise post-exercise muscle reconditioning and increase overall protein intake. Additionally, participants were provided with five high protein yoghurt snacks (~ 14 g protein per 170 g serve; Chobani Australia Pty Ltd, Victoria, Australia) per week to encourage increased protein intake between main meals. Participants were also encouraged to avoid eating energy-dense discretionary foods (e.g., confectionary, prepackaged meals) and to consume no more than two standard drinks of alcohol in one sitting during the exercise training and detraining periods. Dietary intake was monitored weekly by obtaining electronic diet records from participants using either the EasyDietDiary™ or MyFitnessPal™ smartphone application. All dietary intake data was analysed using FoodWorks 8® (Xyris Software Pty Ltd, Australia) for daily averages of energy (kJ·d⁻¹), protein, carbohydrate, and fat (g·kg BW⁻¹ for all macronutrients) for the entire duration of exercise training and detraining periods. Further details can be found in **Appendix A**.

2.3.13 Biochemical and histochemical analyses

2.3.13.1 Immunohistochemistry

A cryostat (Leica CM1850, Leica Biosystems, Victoria, Australia) was used to obtain serial muscle cross sections (7 μ m) which were fixed to specimen slides (SuperFrost Plus, ThermoFisher Scientific, Victoria, Australia), dried at room temperature for 30-60 min and stored at -80 °C until analysis. Slides were fixed in 2% formaldehyde (4% formaldehyde

solution, Merck & Co, Darmstadt, Germany) for 10 min and washed in phosphate-buffered solution (PBS) for 5 min. The PBS was removed and washed in phosphate-buffered solution with Tween (PBST) for 5 min. The PBST was removed and the muscle sections were blocked in a solution containing 2% bovine serum albumin (BSA) in PBS, 5% foetal bovine serum, 0.2% Triton X-100, 0.1% sodium azide and 5% goat serum for 90 min. The blocking solution was removed, and sections were incubated in a primary antibody against laminin (rabbit-anti-human, 1:250 [i.e. one-part antibody in 249 parts 1% BSA], ab11575, Abcam, United Kingdom, RRID:AB_298179) overnight at 4 °C. The following morning, the primary antibody was removed and slides were washed in PBST (3 x 5 min) and incubated in secondary antibody (goat-anti-rabbit, 1:500, Alexa Fluor 488, Life Technologies, California, USA, RRID:AB_2633280) for 120 min at room temperature. Sections were then washed in PBST (3 x 5 min), fixed in 2% PFA for 5 min, washed again in PBST (2 x 5 min), blocked with 10% GS in PBS for 90 min and then incubated in a primary antibody against myosin heavy chain slow (MHCI; mouse-anti-human, 1:2, isoform A4.951, DHSB, Iowa, USA, RRID:AB_528385) overnight at 4 °C. The following morning, sections were washed three times in PBST (3 x 5 min), incubated in secondary antibody (goat-anti-mouse, 1:500, Alexa Fluor 488, Life Technologies, California, USA, RRID:AB_2633275) for 120 min at room temperature and washed again in PBST (3 x 5 min) before being air dried in the dark for 2 min. One drop of fluorescent mounting medium (ProLong™ Diamond Antifade Mountant, Life Technologies, California, USA) was then applied to each section and slides were stored at -20 °C until imaging.

A microscope with a high-resolution fluorescent camera attached was used to view slides. All images were captured through the 20× objective using associated software (EVOS FL Auto 2 cell imaging system, Invitrogen, California, USA). In this study, 58 ± 8 and 78 ± 16 type I and II muscle fibers, respectively, were counted at each biopsy/participant/time point for

CSA. Slides were blinded for both group and time prior to one study researcher (MC) performing all analysis using cell counting software (Fiji, ImageJ Version 2, National Institute of Health, RRID:SCR_002285). The CV between two blinded measurements, completed prior to the commencement of muscle fiber CSA analysis, was 2.7%. Fibers on the periphery of sections were excluded from the analysis. Areas of sections that were affected by freeze fracture artefact or contained longitudinally oriented fibers were excluded from the analysis. If <50 fibers in total were counted at a time point, all muscle fiber CSA data for that participant at that time point was excluded from the analysis. In this study, 31 participants (ENT: n=11, HIIT: n=10, RET: n=10) were included for muscle fiber CSA analysis.

2.3.13.2 Blood analyses

Frozen plasma aliquots were thawed on ice and plasma glucose concentrations were determined in duplicate using a biochemistry analyser (YSI 2900, YSI Life Sciences, Yellow Springs, OH, USA), with a CV of 0.6%. Plasma insulin concentrations were determined in duplicate using an enzyme-linked immunosorbent assay (ELISA; 80-INSHU-E01.1, Abnova Corporation, Taipei, Taiwan), with a CV of 3.2%. Updated homeostatic model assessment of insulin resistance (HOMA2-IR) was calculated using an online calculator by the Diabetes Trials Unit, University of Oxford (<http://www.dtu.ox.ac.uk/homacalculator/index.php>).

2.3.14 Statistical analysis

As no previous studies have compared muscle fiber CSA responses between three different types of single-mode exercise training in human skeletal muscle, sample size was determined a priori (G*Power, Version 3.1) using previous literature assessing muscle fiber CSA changes in response to endurance or resistance exercise training (Farup *et al.*, 2012) with the following inputs: two tailed, effect size (d) = 1.3, α = 0.05 and power = 0.80. Statistical

analyses were performed using SPSS software (Version 25, IBM, USA). Data normality was assessed prior to statistical analysis by assessing skewness, kurtosis and results from Shapiro-Wilk tests. Linear mixed effect models (LME), with subject for random intercept, were used to determine main effects of fixed factors: *time* (Pre, Post, DT), *group* (ENT, HIIT, RET), and interaction (*time* $\times$ *group*) from which residuals were plotted on a histogram to inspect data distribution. For muscle biopsy data, *fiber type* (i.e., type I and II) was included as a third fixed factor in the LME where muscle fiber CSA was the dependent variable.

Where significant main effects were observed (i.e., *time*, *group* or *fiber type*), post hoc comparison with Bonferroni correction was used to locate differences. When significant interaction effects were observed, post hoc comparison with Bonferroni corrections was used to determine within and/or between group differences. Significance was accepted at $P < 0.05$. All data in tables are presented as mean $\pm$ standard deviation. All data in figures are presented as mean and individual participant responses.

2.4 RESULTS

2.4.1 Participant characteristics and dietary intake

At baseline, there were no significant differences in age, body mass index (**Table 2.1**), habitual energy or macronutrient intake between groups (**Table 2.2**). A significant main effect of *time* ($P < 0.001$) was observed for protein intake relative to body mass. Post-hoc comparisons showed that protein intake relative to body mass increased significantly during exercise training in all groups ($P < 0.001$) and remained unchanged during the period of detraining. A significant main effect of *time* ($P = 0.007$) was observed for carbohydrate intake relative to body mass. After detraining, carbohydrate intake relative to body mass decreased significantly compared to baseline in all groups ($P = 0.005$).

Table 2.1 Baseline participant characteristics (Pre) and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

	ENT			HIIT			RET			Main effects (<i>P</i>)		
	Pre	Post	DT	Pre	Post	DT	Pre	Post	DT	Time	Group	Time × group interaction
<i>Participant characteristics</i>												
Age (y)	38.6 ± 2.3			40.4 ± 3.2			39.6 ± 3.5					
Height (cm)	181.2 ± 9.5			179.2 ± 6.1			182.4 ± 6.1					
Body mass (kg)	95.9 ± 15.8	95.9 ± 16.6	95.8 ± 16.4	92.1 ± 11.3	93.1 ± 12.2	93.2 ± 12	93.9 ± 10.4	95.7 ± 11.0	94.4 ± 11.6	0.028	0.835	0.162
BMI (kg·m ⁻²)	29.0 ± 2.6	29.0 ± 3.0	29.0 ± 3.1	28.6 ± 3.0	28.8 ± 3.3	28.8 ± 3.4	28.1 ± 2.2	28.6 ± 2.3	28.5 ± 2.8	0.042	0.888	0.334
<i>Body composition</i>												
Arm lean mass (kg)	8.0 ± 1.3	8.0 ± 1.4	8.0 ± 1.3	7.9 ± 1.0	8.0 ± 1.0	8.0 ± 1.0	8.1 ± 1.0	8.4 ± 1.0 ^a	8.3 ± 1.0	0.040	0.784	0.042
Trunk lean mass (kg)	27.7 ± 2.8	28.0 ± 3.0	27.9 ± 2.8	27.3 ± 2.9	27.4 ± 2.7	27.2 ± 2.5	27.6 ± 3.5	28.1 ± 3.5	27.6 ± 3.3	0.117	0.879	0.609
Body fat %	31.8 ± 6.1	31.4 ± 6.1	31.3 ± 6.4	30.8 ± 3.7	30.4 ± 3.7	30.6 ± 3.7	31.8 ± 3.9	30.9 ± 4.1	31.0 ± 4.1	0.016	0.922	0.074
Muscle thickness (cm)	2.6 ± 0.3	2.6 ± 0.4	2.6 ± 0.3	2.5 ± 0.3	2.8 ± 0.3 ^a	2.7 ± 0.3 ^a	2.5 ± 0.3	2.8 ± 0.3 ^a	2.5 ± 0.3 ^b	<0.001	0.769	<0.001

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. BMI, body mass index. ^a, *P*<0.05 vs Pre within group; ^b, *P*<0.05 vs Post within group. RET at DT: n=10 due to one dropout prior to final measurements.

Table 2.2 Baseline macronutrient intake (Pre) and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

	ENT			HIIT			RET			Main effects (<i>P</i>)		
	Pre	Post	DT	Pre	Post	DT	Pre	Post	DT	Time	Group	Time × group interaction
Energy (kJ·d ⁻¹)	9707 ± 1649	10072 ± 1047	9532 ± 1555	10680 ± 2658	10388 ± 1118	10052 ± 1555	10004 ± 1834	10352 ± 1338	9916 ± 1546	0.281	0.520	0.840
Protein (g·kg BW ⁻¹)	1.3 ± 0.3	1.5 ± 0.2	1.4 ± 0.3	1.2 ± 0.3	1.5 ± 0.2	1.4 ± 0.3	1.1 ± 0.4	1.4 ± 0.2	1.4 ± 0.2	<0.001	0.733	0.130
CHO (g·kg BW ⁻¹)	2.4 ± 0.5	2.3 ± 0.5	2.2 ± 0.6	2.9 ± 1.0	2.6 ± 0.4	2.4 ± 0.6	2.6 ± 0.8	2.6 ± 0.8	2.3 ± 0.7	0.007	0.354	0.671
Fat (g·kg BW ⁻¹)	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.778	0.639	0.960

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. BW, body weight; CHO, carbohydrate; d, day.

2.4.2 Body composition

At baseline, there were no significant differences in body mass (BM), total lean mass (LM), appendicular (ALM), leg lean mass (LLM) or body fat percentage between groups (**Figure 2.3** and **Table 2.1**).

A significant *time* $\times$ *group* interaction effect ($P=0.023$) was observed for LM (**Figure 2.3A**). In response to exercise training, LM increased significantly for both RET ($+2.0 \pm 1.0$ kg, $P<0.001$) and HIIT ($+1.0 \pm 1.2$ kg, $P=0.011$), but not for ENT. After detraining, LM remained unchanged compared to post-exercise training for both RET and HIIT. After detraining, LM remained significantly elevated compared to baseline for both RET ($+1.0 \pm 1.2$ kg, $P=0.020$) and HIIT ($+1.0 \pm 1.3$ kg, $P=0.010$), but not for ENT.

A significant *time* $\times$ *group* interaction effect ($P=0.033$) was observed for ALM (**Figure 2.3B**). In response to exercise training, ALM increased significantly for both RET ($+1.3 \pm 1.2$ kg, $P<0.001$) and HIIT ($+0.8 \pm 0.8$ kg, $P=0.004$), but not for ENT. After detraining, ALM remained unchanged compared to post-exercise training for both RET and HIIT. After detraining, ALM remained significantly elevated compared to baseline for RET ($+1.0 \pm 1.0$ kg, $P=0.001$) and HIIT ($+1.0 \pm 0.8$ kg, $P=0.001$), but not for ENT.

A significant main effect of *time* ($P<0.001$) was observed for LLM, with an increase in LLM in response to exercise training in all groups ($P<0.001$), that was maintained after detraining (**Figure 2.3C**).

A significant main effect of *time* ($P=0.016$) was observed for body fat percentage (**Table 2.1**). Post-hoc comparisons showed a significant decrease for body fat percentage after exercise training ($P=0.014$), that was maintained following detraining.

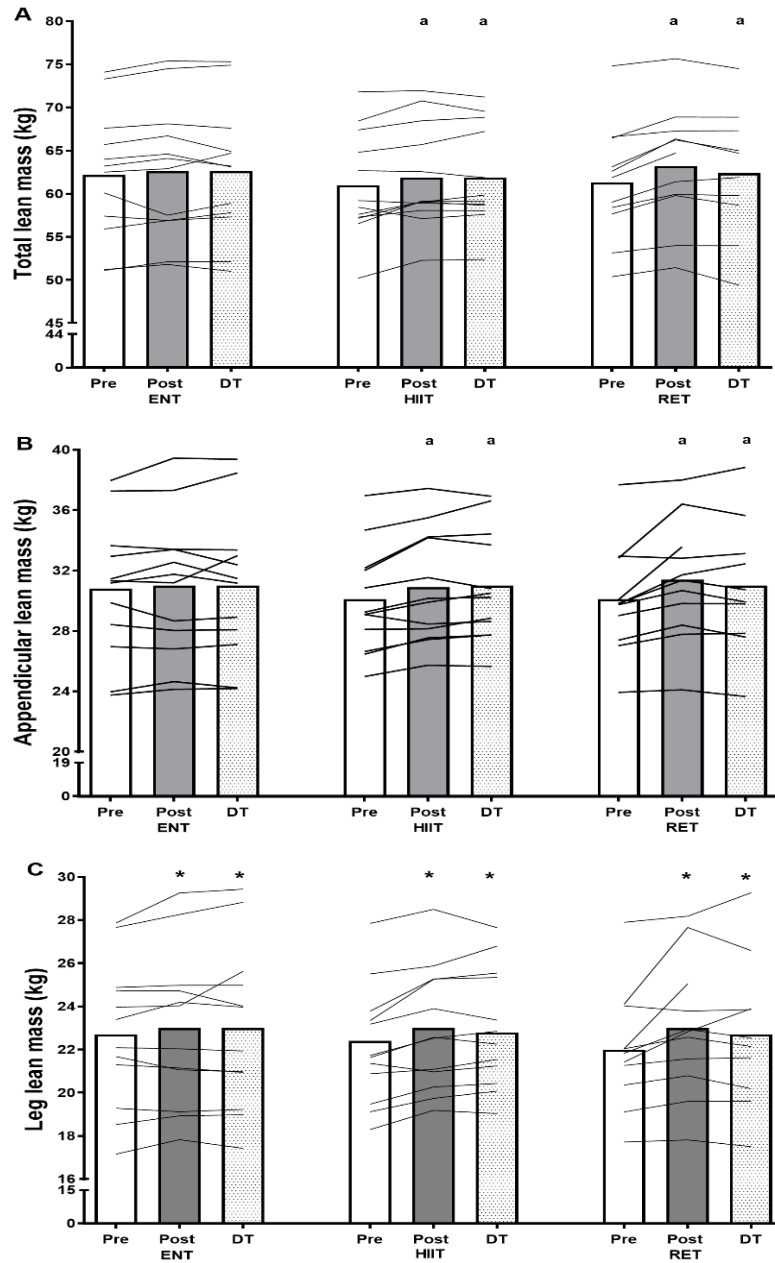


Figure 2.3 Baseline (Pre) total (A), appendicular (B) and leg (C) lean mass and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and individual responses. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. ^a, $P < 0.05$ vs Pre within group; ^b, $P < 0.05$ vs Post within group; *, main effect of time ($P < 0.05$) vs Pre. RET at DT: $n = 10$.

2.4.3 1RM muscle strength

There were no significant differences at baseline for 1RM leg press, leg extension or bench press muscle strength between groups (**Figure 2.4**). A significant *time* $\times$ *group* interaction effect ($P<0.001$) was observed for 1RM leg press muscle strength (**Figure 2.4A**). In response to exercise training, 1RM leg press muscle strength increased significantly in all groups (RET: $+70 \pm 31$ kg, $P<0.001$; HIIT: $+27 \pm 19$ kg, $P<0.001$; ENT: $+16 \pm 16$ kg, $P=0.026$). After detraining, 1RM leg press muscle strength increased significantly compared to post-exercise training for ENT ($+16 \pm 18$ kg, $P=0.008$), but remained unchanged for both RET and HIIT. After detraining, 1RM leg press muscle strength remained significantly elevated compared to baseline (all $P<0.001$).

A significant *time* $\times$ *group* interaction effect ($P<0.001$) was observed for change in relative 1RM leg press muscle strength (**Figure 2.4B**). In response to exercise training, relative 1RM leg press muscle strength increased significantly in all groups (RET: $+0.7 \pm 0.3$ kg·kg BM⁻¹, $P<0.001$; HIIT: $+0.3 \pm 0.2$ kg·kg BM⁻¹, $P=0.001$; ENT: $+0.2 \pm 0.2$ kg·kg BM⁻¹, $P=0.028$). After detraining, relative 1RM leg press muscle strength increased significantly for ENT compared to post-exercise training ($+0.2 \pm 0.2$ kg·kg BM⁻¹, $P=0.013$), but HIIT and RET remained unchanged. After detraining, relative 1RM leg press muscle strength remained elevated compared to baseline in all groups (RET: $+0.6 \pm 0.3$ kg·kg BM⁻¹, $P<0.001$; HIIT: $+0.3 \pm 0.3$ kg·kg BM⁻¹, $P<0.001$; ENT: $+0.4 \pm 0.3$ kg·kg BM⁻¹, $P<0.001$).

A significant *time* $\times$ *group* interaction effect ($P=0.022$) was observed for 1RM leg extension muscle strength (**Figure 2.4C**). In response to exercise training, 1RM leg extension muscle strength increased significantly for both RET ($+23 \pm 11$ kg, $P<0.001$) and HIIT ($+11 \pm 11$ kg, $P=0.005$) while there was a trend for and increase for ENT ($+8 \pm 12$ kg, $P=0.064$). After detraining, 1RM leg extension muscle strength remained unchanged in all groups compared to

post-exercise training. After detraining, 1RM leg extension muscle strength remained significantly elevated compared to baseline (all $P<0.05$).

A significant main effect of *time* ($P<0.001$) was observed for change in relative 1RM leg extension muscle strength (**Figure 2.4D**). Post-hoc comparisons showed that 1RM leg extension muscle strength increased significantly after exercise training in all groups ($P<0.001$) and remained elevated after detraining.

A significant *time* $\times$ *group* interaction effect ($P=0.001$) was observed for 1RM bench press muscle strength (**Figure 2.4E**). In response to exercise training, 1RM bench press muscle strength increased significantly for both RET ($+10 \pm 4$ kg, $P<0.001$) and HIIT ($+5 \pm 6$ kg, $P=0.034$), but not for ENT. After detraining, 1RM bench press muscle strength remained unchanged compared to post-exercise training for both RET and HIIT. After detraining, 1RM bench press muscle strength remained significantly elevated compared to baseline for RET ($+15 \pm 13$ kg, $P<0.001$), but not for HIIT or ENT.

A significant *time* $\times$ *group* interaction effect ($P=0.001$) was observed for change in relative 1RM bench press muscle strength (**Figure 2.4F**). In response to exercise training, relative 1RM bench press muscle strength increased significantly for RET ($+0.10 \pm 0.03$ kg·kg BM^{-1} , $P=0.001$) and there was a trend for an increase for HIIT ($P=0.057$). After detraining, relative 1RM bench press muscle strength remained unchanged compared to post-exercise training for RET. After detraining, relative 1RM bench press muscle strength remained elevated compared to baseline for RET ($+0.15 \pm 0.13$ kg·kg BM^{-1} , $P<0.001$), but not for HIIT or ENT.

A significant *time* $\times$ *group* interaction effect ($P=0.001$) was observed for the sum of all 1RM. In response to exercise training, the sum of all 1RM increased significantly in all groups (RET: $+90 \pm 38$ kg, $P<0.001$; HIIT: $+41 \pm 23$ kg, $P=0.001$; ENT: $+23 \pm 21$ kg, $P=0.002$). After detraining, the sum of all 1RM remained unchanged compared to post-exercise training

in all groups. After detraining, the sum of all 1RMs remained significantly elevated compared to baseline (all $P<0.05$).

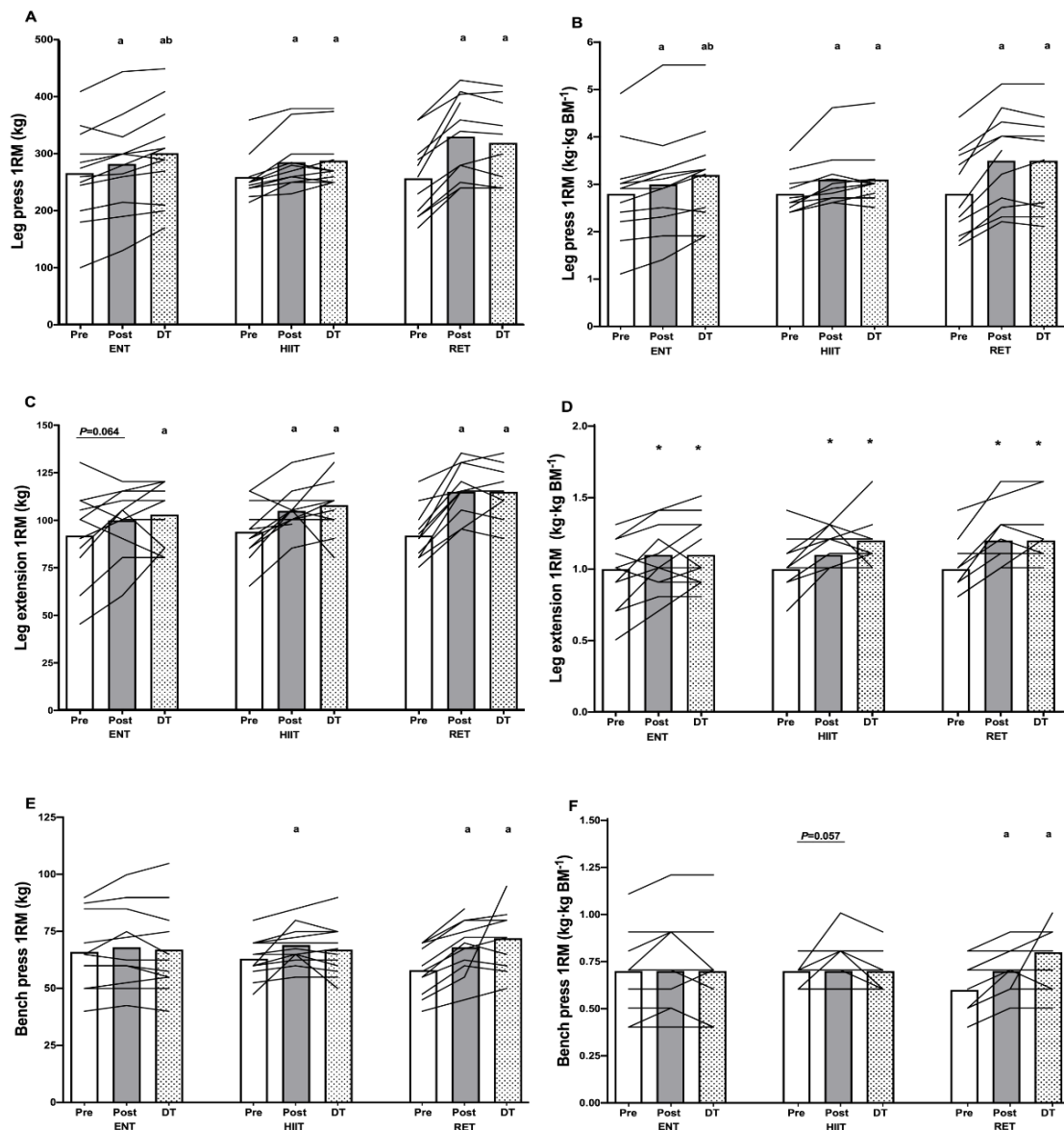


Figure 2.4 Baseline (Pre) absolute (A, C, E) and relative (B, D, F) 1RM muscle strength and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and individual responses. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. 1RM, one-repetition maximum. ^a, $P<0.05$ vs Pre within group; ^b, $P<0.05$ vs Post within group. ENT, bench press: $n=11$; HIIT, leg press at Post: $n=11$; RET at DT: $n=10$.

2.4.4 VO₂peak and maximal aerobic power

At baseline, there were no significant differences in VO₂peak or maximal aerobic power (MAP) between groups (**Figure 2.5**). A significant *time* × *group* interaction effect ($P=0.001$) was observed for VO₂peak (**Figure 2.5A**). In response to exercise training, VO₂peak increased significantly for both HIIT ($+0.4 \pm 0.2 \text{ L}\cdot\text{min}^{-1}$, $14 \pm 7\%$, $P<0.001$) and ENT ($+0.3 \pm 0.3 \text{ L}\cdot\text{min}^{-1}$, $11 \pm 11\%$, $P<0.001$), but not for RET. After detraining, VO₂peak decreased significantly compared to post-exercise training for HIIT ($-0.2 \pm 0.1 \text{ L}\cdot\text{min}^{-1}$, $-6 \pm 4\%$, $P=0.005$) and there was a trend for a decreased VO₂peak for ENT ($-0.1 \pm 0.2 \text{ L}\cdot\text{min}^{-1}$, $-4 \pm 5\%$, $P=0.055$). After detraining, VO₂peak remained elevated compared to baseline for both HIIT ($+0.2 \pm 0.2 \text{ L}\cdot\text{min}^{-1}$, $8 \pm 6\%$, $P=0.001$) and ENT ($+0.2 \pm 0.2 \text{ L}\cdot\text{min}^{-1}$, $6 \pm 7\%$, $P=0.009$), but not for RET.

A significant *time* × *group* interaction effect ($P=0.002$) was observed for change in relative VO₂peak (**Figure 2.5B**). In response to exercise training, relative VO₂peak increased significantly for HIIT ($+4.1 \pm 2.7 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P<0.001$) and ENT group ($+3.5 \pm 2.9 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P<0.001$), but not for RET. After detraining, relative VO₂peak decreased compared to post-exercise training for HIIT ($-1.9 \pm 1.7 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P=0.029$) and ENT ($-1.7 \pm 1.7 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P=0.024$). After detraining, relative VO₂peak remained elevated compared to baseline for HIIT ($+2.3 \pm 2.8 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P=0.024$) and ENT ($+1.8 \pm 1.9 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P=0.003$), but not for RET.

A significant *time* × *group* interaction effect ($P<0.001$) was observed for MAP (**Figure 2.5C**). In response to exercise training, MAP increased significantly for both HIIT ($+30 \pm 9 \text{ W}$, $P<0.001$) and ENT ($+26 \pm 14 \text{ W}$, $P<0.001$), but not for RET. After detraining, MAP decreased significantly for HIIT ($-17 \pm 11 \text{ W}$, $P=0.001$) compared to post-exercise training but remained unchanged for ENT. After detraining, MAP remained significantly elevated compared to baseline for both HIIT ($+13 \pm 14 \text{ W}$, $P<0.001$) and ENT ($+23 \pm 13 \text{ W}$, $P<0.001$), but not for RET.

A significant *time* $\times$ *group* interaction effect ($P<0.001$) was observed for change in relative MAP (**Figure 2.5D**). In response to exercise training, relative MAP increased significantly for HIIT ($+0.4 \pm 0.2 \text{ W}\cdot\text{kg}^{-1}$, $P<0.001$) and ENT ($+0.3 \pm 0.2 \text{ W}\cdot\text{kg}^{-1}$, $P<0.001$), but not for RET. After detraining, relative MAP decreased significantly compared to post-exercise training for HIIT ($-0.2 \pm 0.2 \text{ W}\cdot\text{kg}^{-1}$, $P=0.001$), but remained unchanged for ENT. After detraining, relative MAP remained elevated compared to baseline for both ENT ($+0.2 \pm 0.2 \text{ W}\cdot\text{kg}^{-1}$, $P<0.001$) and HIIT ($+0.1 \pm 0.2 \text{ W}\cdot\text{kg}^{-1}$, $P=0.034$), but not for RET.

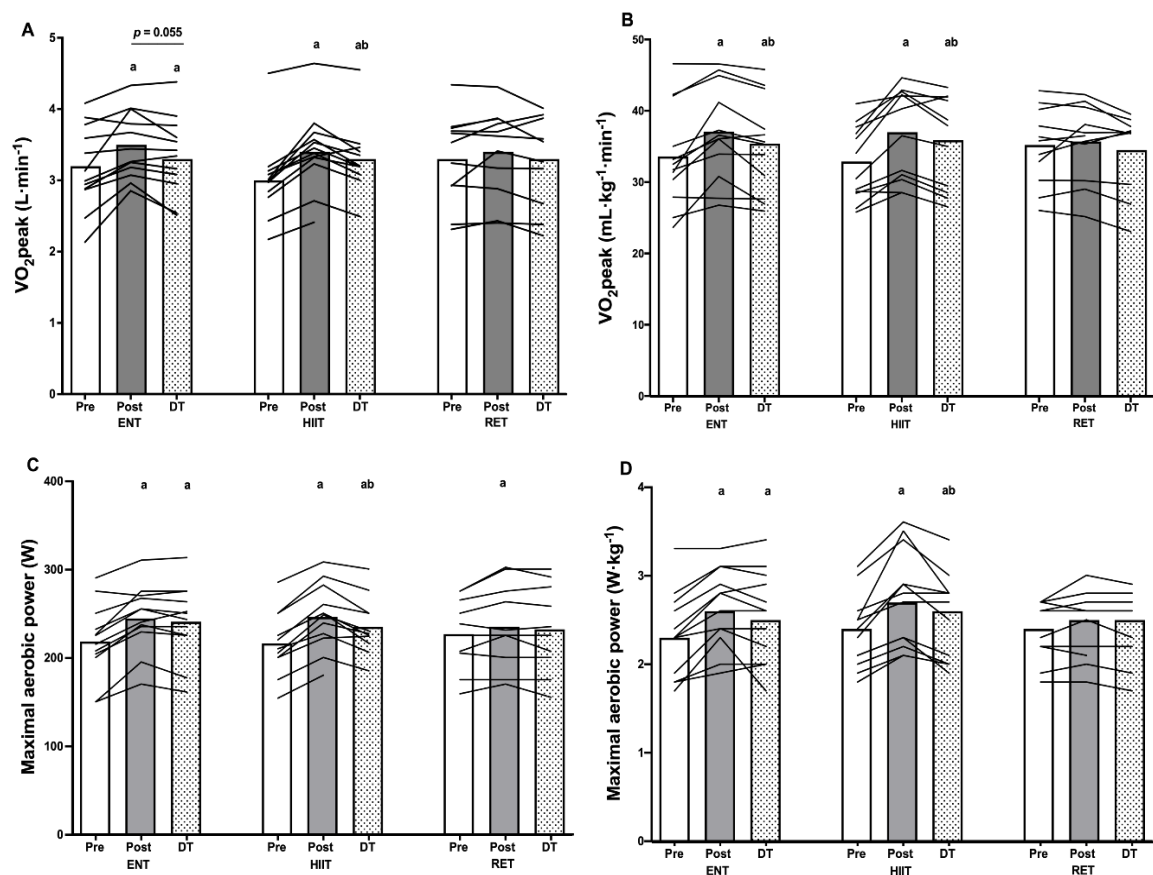


Figure 2.5 Baseline (Pre) $\text{VO}_{2\text{peak}}$ (A, B) and maximal aerobic power (C, D) and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and individual responses. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. W, watt. ^a, $P<0.05$ vs Pre within group; ^b, $P<0.05$ vs Post within group. HIIT at DT: $n=11$; RET at DT: $n=10$

2.4.5 Muscle fiber characteristics

At baseline, there were no significant differences in type I or II muscle fiber cross-sectional area (CSA) between groups (**Table 2.3**). There was no significant *time* $\times$ *group* $\times$ *fiber type* interaction effect for muscle fiber CSA. A significant main effect of *time* was observed for type I and II muscle fiber CSA in all groups (all, $P=0.007$). In response to exercise training, type I and type II muscle fiber CSA increased significantly ($P=0.006$) in all groups. After detraining, type I and II muscle fiber CSA remained unchanged compared to post-exercise training in all groups.

At baseline, there were no significant differences in the proportion of type I or type II fibers between groups (**Table 2.3**). A significant main effect of *time* was observed for both type I and II fiber type percentages (both $P<0.05$), however, no significant differences between time points could be detected in all groups.

Table 2.3 Baseline *vastus lateralis* muscle fiber characteristics (Pre) and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

	ENT			HIIT			RET			Main effects (<i>P</i>)		
	Pre	Post	DT	Pre	Post	DT	Pre	Post	DT	Time	Group	Time × group interaction
Type I CSA (μm ²)	3672 ± 776	4245 ± 1309	4268 ± 1336	3672 ± 698	4611 ± 1364	3616 ± 555	4412 ± 1329	4345 ± 962	3837 ± 872	0.007	0.760	0.313
Type II CSA (μm ²)	4269 ± 943	4949 ± 2060	4804 ± 1548	3423 ± 719	3960 ± 774	3690 ± 961	3479 ± 865	4222 ± 867	3552 ± 456	0.007	0.760	0.313
Type I %	46 ± 12	41 ± 10	49 ± 14	40 ± 18	39 ± 21	48 ± 18	36 ± 10	44 ± 10	40 ± 8	0.034	0.923	0.383
Type II %	54 ± 12	59 ± 10	51 ± 14	60 ± 18	61 ± 21	52 ± 18	64 ± 10	56 ± 10	60 ± 8	0.034	0.923	0.383

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. CSA, muscle fiber cross-sectional area. Fiber type % is ENT: n=11; HIIT: n=10; RET: n=10.

2.4.6 Muscle thickness

At baseline, there were no significant differences in muscle thickness (MT) between groups (**Table 2.1**). A significant *time* $\times$ *group* interaction effect ($P<0.001$) was observed for muscle thickness. In response to exercise training, MT increased significantly for both RET ($+0.3 \pm 0.2$ cm, $P<0.001$) and HIIT ($+0.3 \pm 0.2$ cm, $P<0.001$), but not for ENT. After detraining, MT decreased significantly compared to post-exercise training for RET (-0.3 ± 0.1 cm, $P<0.001$), but remained unchanged for HIIT. After detraining, MT remained significantly elevated compared to baseline for HIIT ($+0.2 \pm 0.2$ cm, $P<0.001$), but returned to pre-exercise training levels for RET.

2.4.7 Resting energy expenditure & oral glucose tolerance test

At baseline, there were no significant differences in REE between groups (**Table 2.4**). REE did not change after exercise training or detraining in all groups.

At baseline, there were no differences between groups for any measurements derived from the OGTT (**Table 2.4**). A significant *time* $\times$ *group* interaction effect ($P=0.001$) was observed for fasting plasma glucose. In response to exercise training, fasting plasma glucose increased significantly for RET ($+0.4 \pm 0.6$ mmol·L⁻¹, $P=0.001$), but not HIIT or ENT. After detraining, fasting plasma glucose decreased significantly compared to post-exercise training for RET (-0.4 ± 0.5 mmol·L⁻¹, $P=0.002$). Total glucose area under the curve did not change after exercise training or detraining in all groups.

A significant main effect of *time* ($P=0.035$) was observed for fasting plasma insulin. Post-hoc comparisons showed a significant decrease for fasting plasma insulin after detraining ($P=0.038$) compared to baseline. A trend for a *time* $\times$ *group* interaction effect was observed for total insulin area under the curve ($P=0.061$). HOMA2-IR did not change after exercise training or detraining in all groups.

Table 2.4 Baseline (Pre) resting energy expenditure and oral glucose tolerance measurements and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

	ENT			HIIT			RET			Main effects (<i>P</i>)		
	Pre	Post	DT	Pre	Post	DT	Pre	Post	DT	Time	Group	Time × group interaction
<i>Resting metabolic rate test</i>												
Resting energy expenditure (kcal)	1974 ± 210	1968 ± 210	1926 ± 198	1955 ± 210	1941 ± 210	1922 ± 204	2056 ± 210	2014 ± 210	2028 ± 190	0.349	0.486	0.924
<i>Oral glucose tolerance test</i>												
Fasting plasma glucose (mmol·L ⁻¹)	5.4 ± 0.5	5.2 ± 0.5	5.5 ± 0.8	5.1 ± 0.3	5.0 ± 0.3	5.1 ± 0.3	5.2 ± 0.9	5.5 ± 0.5 ^a	5.1 ± 0.9 ^b	0.848	0.501	0.001
AUC _{total} glucose (mmol·h ⁻¹ ·L ⁻¹)	893 ± 193	824 ± 131	899 ± 179	726 ± 99	721 ± 108	731 ± 154	874 ± 165	798 ± 139	773 ± 146	0.085	0.273	0.113
Fasting plasma insulin (mIU·L ⁻¹)	7.4 ± 5.9	6.0 ± 5.1	6.7 ± 5.4	5.3 ± 2.7	5.5 ± 2.8	4.5 ± 2.4	9.8 ± 7.7	8.1 ± 6.6	7.9 ± 4.7	0.035	0.349	0.246
AUC _{total} insulin (mmol·h ⁻¹ ·L ⁻¹)	6098 ± 3810	6096 ± 6496	7136 ± 6297	4563 ± 2063	3955 ± 1883	4194 ± 2118	9277 ± 5751	7271 ± 4196	7239 ± 4819	0.129	0.097	0.061
HOMA2-IR	1.0 ± 0.7	0.8 ± 0.7	0.9 ± 0.7	0.7 ± 0.3	0.7 ± 0.4	0.6 ± 0.3	1.2 ± 1.0	1.1 ± 0.9	1.1 ± 0.6	0.133	0.207	0.559

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. AUC, area under the curve; HOMA2-IR, homeostatic model assessment of insulin resistance. ^a, *P*<0.05 vs Pre within group; ^b, *P*<0.05 vs Post within group.

RET at DT: n=10.

2.4.8 Physical activity

At baseline, there were no significant differences in physical activity measurements between groups (**Table 2.5**). A trend for a main effect of *time* was observed for daily step count ($P=0.067$). A significant main effect of *time* was observed for the percentage of the day spent moving ($P=0.011$) and standing ($P=0.023$). Post-hoc comparisons showed a reduction in the percentage of the day spent moving ($P=0.011$) and standing ($P=0.020$) during detraining compared to week four of exercise training.

A significant main effect of *time* was observed for percentage of the day spent sitting ($P=0.011$). Post-hoc comparisons showed an increase in the percentage of the day spent sitting during detraining compared to week four of exercise training ($P=0.011$).

A significant *time* $\times$ *group* interaction effect ($P<0.001$) was observed for the percentage of the day spent cycling. At week four of exercise training, the percentage of the day spent cycling increased significantly compared to baseline for ENT ($+1 \pm 1\%$, $P<0.001$), but not for HIIT or RET. At week four of exercise training, the percentage of the day spent cycling was significantly greater for ENT compared to HIIT ($+1 \pm 1\%$, $P=0.010$) and RET ($+2 \pm 1\%$, $P<0.001$).

Table 2.5 Baseline physical activity (Pre) and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

	ENT			HIIT			RET			Main effects (<i>P</i>)		
	Pre	Week 4	DT	Pre	Week 4	DT	Pre	Week 4	DT	Time	Group	Time × group interaction
Steps (steps·d ⁻¹)	8907 ± 2163	11192 ± 3495	9072 ± 2699	9385 ± 1324	10409 ± 2847	9672 ± 2322	10134 ± 3180	9875 ± 1787	9560 ± 2296	0.067	1.000	0.500
Moving (%·d ⁻¹)	15 ± 8	18 ± 6	13 ± 4	14 ± 2	16 ± 4	14 ± 5	14 ± 4	15 ± 3	13 ± 3	0.011	0.862	0.388
Stepping (%·d ⁻¹)	14 ± 7	16 ± 7	13 ± 3	13 ± 2	15 ± 3	14 ± 5	14 ± 3	15 ± 3	13 ± 3	0.048	0.991	0.795
Cycling (%·d ⁻¹)	1 ± 1	2 ± 1 ^a	0 ± 1 ^b	0 ± 1	1 ± 0*	0 ± 1	0 ± 0	0 ± 0*	0 ± 0	<0.001	0.023	<0.001
Standing (%·d ⁻¹)	26 ± 9	24 ± 10	22 ± 6	24 ± 9	26 ± 8	22 ± 7	26 ± 11	31 ± 10	27 ± 11	0.023	0.613	0.261
Sitting (%·d ⁻¹)	60 ± 15	59 ± 13	65 ± 8	64 ± 11	59 ± 9	64 ± 11	60 ± 13	55 ± 12	60 ± 12	0.011	0.772	0.747
Total time awake (h·d ⁻¹)	14 ± 4	14 ± 3	15 ± 3	14 ± 2	14 ± 2	13 ± 1	15 ± 2	14 ± 2	15 ± 2	0.917	0.627	0.732

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. d, day. ^a, *P*<0.05

vs Pre within group; ^b, *P*<0.05 vs Post within group; *, *P*<0.05 vs ENT at week 4. Moving = stepping + cycling.

2.5 DISCUSSION

In the current study, short-term HIIT induced widespread changes in whole-body physical fitness and skeletal muscle adaptation as demonstrated by increases in peak aerobic capacity, lean mass, muscle thickness and muscle strength. While the exercise training-induced gains in lean mass with RET and HIIT were maintained following short-term detraining, improvements in aerobic capacity after HIIT and ENT did not persist.

2.5.1 Exercise training responses

2.5.1.1 Muscle strength & aerobic capacity

Physical fitness prior to surgery is an independent predictor of post-operative morbidity and mortality (Pouwels *et al.*, 2016). Pre-operative exercise training is one tool that can enhance physical fitness and better prepare an individual for subsequent surgery. However, pre-operative exercise training programming requires optimisation (e.g., single- vs dual-mode) to meet the individual needs of various clinical populations within a short time-frame (Topal *et al.*, 2019). After six weeks of exercise training, all exercise modalities increased whole-body muscle strength (i.e., sum of all 1RMs), although exercise training-induced increases in aerobic capacity were only induced after HIIT and ENT.

Only one other study has directly compared the effects of ENT, HIIT and RET on muscle strength and aerobic capacity in middle-aged adults (Schjerve *et al.*, 2008). In that study, 12 weeks of RET increased 1RM leg press muscle strength (+25%) but walking/running HIIT and ENT did not (Schjerve *et al.*, 2008). In contrast, results from the current study revealed an increase in 1RM leg press muscle strength with cycling HIIT (+11%) and ENT (+8%). While it is difficult to explain why the shorter HIIT and ENT protocols (i.e., six vs 12 weeks) increased 1RM muscle strength, key differences were apparent in study designs between the current study and that of Schjerve *et al.* (2008). Firstly, the exercise programme in the current study involved a progressive overload where all exercise groups were retested halfway through the training

intervention to readjust training zones. Secondly, dietary protein intake was elevated ($\sim 1.4 \text{ g} \cdot \text{kg} \text{ BW}^{-1} \cdot \text{d}^{-1}$) to help augment anabolic adaptations to exercise training. Finally, all exercise training sessions were supervised in the laboratory to monitor first-hand training technique and ensure appropriate training intensity. Collectively, increases in muscle strength with short-term aerobic-based exercise training may be dependent on incorporating supervised training programs that provide appropriate training intensity/progressive overload and with supportive nutritional measures. However, an important limitation to this inference is that the current study only recruited males. Whether females could similarly increase muscle strength with short-term HIIT, particularly in light of substantial differences in hormonal milieu between sexes in middle-aged adulthood, remains an area of further investigation. Additionally, the potential of a 'learned effect' that may explain the current strength results cannot be ruled out. Thus, future studies that incorporate appropriate strength testing familiarisation sessions preceding short-term HIIT and ENT training are warranted.

In the current study, HIIT and ENT increased peak aerobic capacity (+14% and +11%, respectively), but not after RET, in support of previous reports in middle-aged adults (Bagley *et al.*, 2016; Dohlmann *et al.*, 2018; Keating *et al.*, 2014; Matsuo *et al.*, 2015; Nybo *et al.*, 2010). Increased aerobic capacity of a similar magnitude ($\sim 10\%$) to that seen after HIIT and ENT have been reported following 12 weeks of RET in middle-aged adults (Schjerve *et al.*, 2008). Therefore, the RET programme used in the current study may not be the most suitable where short-term RET-induced improvements in aerobic capacity are desired. Alternatively, circuit-based RET involving whole-body movement that induces greater stress on the cardiovascular system, while also increasing muscle strength, may be a better option for enhancement of both components of physical function. Although participants in the current study were not pre-operative patients, these results demonstrate that short-term HIIT and ENT can lead to improved muscle strength following short-term exercise training. Short-term cycling-based exercise training (e.g., ENT or HIIT) may be of use in clinical scenarios where

the surgery patient cannot or does not wish to participate in strength-based exercise training (e.g. physical restrictions, access to equipment, instructor availability).

2.5.1.2 Lean mass, muscle thickness and muscle fiber size

In many populations, reduced functional capacity and skeletal muscle mass is a predictor of unfavourable post-operative outcomes (Levolger *et al.*, 2015; Moskven *et al.*, 2018; Ratnayake *et al.*, 2018). Thus, pre-operative exercise training-induced increases in skeletal muscle mass can better prepare the patient for surgery and the ensuing recovery period (Scheede-Bergdahl *et al.*, 2019). There is a paucity of information regarding lean mass responses following different types of short-term exercise training in both pre-surgery patients and healthy middle-aged adults. In the current study, the largest increase in lean mass was induced by RET (+2 kg), although HIIT also significantly increased lean mass (+1 kg), while there was no change observed after ENT. While this increase in lean mass with HIIT was statistically significant, it must be noted that this increase was very similar to the CV of the densitometer used to obtain lean mass measurements (i.e., 1.6 vs 1.5%). As such, this increase in lean mass following HIIT should be interpreted with caution. Robinson *et al.* (2017) reported increases in fat-free mass (FFM) in young and older adults following 12 weeks of RET or HIIT (~2 kg and ~1 kg, respectively). Despite the shorter exercise training protocols in the present study, similar changes in FFM (data not shown) were observed after RET and HIIT. Unlike the current study, Robinson *et al.* (2017) did not control for dietary protein intake. Indeed, in the current study participants met a protein target (~1.4 g·kg BW⁻¹·d⁻¹) recommended to promote and maintain muscle growth while exercise training (Jager *et al.*, 2017) as part of a free-living eating plan that may have augmented gains in lean mass. Without a non-protein supplemented exercise training group (e.g., ~1.0 g·kg BW⁻¹·d⁻¹) the contribution of increased protein intake to observed gains in lean mass cannot be evaluated.

Six weeks of RET and HIIT, but not ENT, increased *vastus lateralis* muscle thickness (+11% and 10%, respectively). A previous study reported that 12 weeks of RET and ENT increased *vastus lateralis* muscle thickness (+14% and 10%, respectively) in young recreationally active men consuming a high-protein diet (2 g/kg BW⁻¹·d⁻¹) (Shamim *et al.*, 2018a). Notably, ENT comprised interval-style exercise sessions in the final month of the programme in that study (Shamim *et al.*, 2018a). Results from the current study show that improvements of a similar magnitude are attainable after short-term RET and HIIT in middle-aged men. However, as ultrasound measures occurred ~24-48 hours following muscle biopsies in all participants due to unavoidable logistical reasons, the possibility that local oedema/swelling in the *vastus lateralis* may have affected muscle thickness measurements cannot be ruled out. Taken together, these findings suggest that higher-intensity intermittent exercise (aerobic- or resistance-based) may be an important consideration where exercise training-induced increases in muscle mass/thickness are required in a relatively short period of time (i.e., six weeks) in middle-aged men.

This is the first study to report changes in muscle fiber size following ENT, HIIT and RET in middle-aged men. De Souza *et al.* (2014) observed muscle fiber hypertrophy (type I and IIa) following eight weeks of RET but not HIIT in young men. As the HIIT intervention in that study involved treadmill running, it is plausible the high eccentric component of this contractile mode (compared to cycling which produces movement primarily concentrically) may have induced greater muscle damage and thus limited its anabolic potential. Indeed, previous work has reported running-based activity to produce greater attenuation of lower-body muscle strength and hypertrophy compared with cycling (Wilson *et al.*, 2012). Farup *et al.* (2012) reported increased type II but not type I muscle fiber size in response to 10 weeks of RET but not ENT (that included one weekly HIIT session) in young, untrained men. In contrast to these studies (de Souza *et al.*, 2014; Farup *et al.*, 2012), results from the current study show short-term cycling exercise training increases type I and II muscle fiber size in middle-aged

men. While the discordance in these findings (de Souza *et al.*, 2014; Farup *et al.*, 2012) compared to the current study are difficult to reconcile considering the similarities in participant training status and mode of ENT, the potential for aerobic-based exercise to increase muscle fiber CSA is an area of research requiring further investigation that may confer health and performance benefits. It must also be acknowledged that the variation in resting fiber CSA among the sedentary middle-aged participants studied may have limited the potential to detect post-exercise training and detraining muscle fiber CSA changes.

The current findings demonstrate that short-term HIIT can concurrently increase aerobic capacity and lean mass, an outcome that would typically be achieved using combined exercise training. As HIIT is a time-efficient intervention and may be perceived as more enjoyable to undertake compared to ENT (Bartlett *et al.*, 2011), HIIT may be appealing to some pre-surgery patients. However, future studies comparing short-term HIIT and combined exercise training in surgery patients are needed to confirm if HIIT can match the beneficial physical effects of combined exercise training.

2.5.1.3 Resting energy expenditure & glucose homeostasis

Fat-free mass, including metabolically active tissue such as skeletal muscle, is a large determinant of resting energy expenditure (REE) (Müller *et al.*, 2002). Increases in surrogates of muscle mass (e.g., lean/fat-free mass) and REE have been reported following different types of prolonged exercise training in some (Poehlman *et al.*, 2002) but not all studies (Alberga *et al.*, 2017; Broeder *et al.*, 1992). In the current study, both RET and HIIT increased lean mass without detectable changes in REE (via RMR measurement). Exercise training did not improve fasting glucose, insulin or respective areas under the curve following a two-hour OGTT, most likely because participants in the current study had good glycaemic control prior to the study.

2.5.2 Detraining responses

2.5.2.1 Muscle strength & aerobic capacity

Short periods (~two weeks) of reduced physical activity induce skeletal muscle deconditioning (Bell *et al.*, 2016; Wall *et al.*, 2013). Exercise training can attenuate catabolic events typically observed during periods of reduced physical activity (Oikawa *et al.*, 2019). However, studies addressing the exercise modality that best preserves physical fitness and skeletal muscle adaptation responses following short-term exercise training cessation are few. In the current study, exercise training-induced gains in muscle strength were maintained following 2.5 weeks of detraining, despite declines in aerobic capacity. Although participants were ambulatory during the detraining period, a reduction in the percentage of the day spent moving and an increase in percentage of the day spent sitting (main effects of time) was observed.

Spence *et al.* (2011) investigated the effects of six weeks of detraining following six months of ENT or RET on aerobic capacity and muscle strength in young men. Detraining resulted in a significant decline in aerobic capacity with ENT despite the maintenance of lower body muscle strength (1RM squat). In contrast, upper (1RM bench press) and lower body muscle strength gains persisted after detraining following the RET intervention (Spence *et al.*, 2011). In the current study, similar patterns for changes in aerobic capacity and muscle strength following detraining with ENT and RET. Declines in $\text{VO}_{2\text{peak}}$ concomitant with loss of capillarisation and mitochondrial enzyme activities have been reported following 2-4 weeks of detraining proceeding short-term aerobic exercise training (Klausen *et al.*, 1981; Wibom *et al.*, 1992). Additionally, loss of plasma volume may have also contributed to observed decreases in aerobic capacity following short-term detraining (Coyle *et al.*, 1986). Overall, results from the current study and previous work (Spence *et al.*, 2011) indicate that short-term and prolonged ENT-induced increases in aerobic capacity are lost rapidly (~2-6 weeks) compared to the time-course for declines in muscle strength in young and middle-aged men.

2.5.2.2 Lean mass, muscle thickness and muscle fiber size

In the current study, exercise training-induced increases in surrogates of whole-body and regional muscle mass after RET and HIIT were maintained following a short period of exercise training cessation similar in duration to the early post-operative period faced by patients who have undergone surgery. As such, short-term single-mode higher-intensity exercise training prior to periods of forced inactivity may provide benefits to physical function, combatting the catabolic effects of reduced physical activity typically observed following inactivity and/or surgery (van der Meij *et al.*, 2017; Wasowicz-Kemps *et al.*, 2009). However, it should also be noted that leg press muscle strength gains were preserved following detraining with ENT. As lower body muscle strength contributes to mobility, it is speculated that different types of single-mode exercise training (albeit to varying magnitudes) are capable of retaining components important for physical function. Whether preservation of lower body muscle strength aligns with improved early post-operative clinical outcomes (e.g., less time in hospital, reduced post-operative complications) in surgery cohorts following different types of short-term exercise training remains to be determined. In the current study, Type I and II muscle fiber CSA was maintained after detraining independent of exercise training modality. However, we must acknowledge that both type I and II fiber CSA were numerically lower than post-exercise training levels after detraining in the RET (type I: -11%, type II: -16%) and HIIT groups (type I: -20%, type II: -10%). While this reduction was not statistically significant likely due to low sample size, it cannot be discounted that the observed decrease with detraining represents a clinically relevant decrease in muscle fiber size.

Although the middle-aged men in the current study are likely to have been more physically active during the detraining period than many post-operative patients, early ambulation is often encouraged where possible following surgery (Gustafsson *et al.*, 2019). Thus, results from the current study provide new information that short-term single-mode higher intensity exercise training can counter some of the catabolic effects in skeletal muscle

induced by short-term reduced physical activity (e.g., decreased muscle mass). Whether single-mode exercise training can benefit muscle adaptation responses shortly before and after surgery needs to be explored in a variety of surgery populations (e.g., elective surgery, oncology).

2.6 CONCLUSION

Six weeks of RET and HIIT but not ENT increased markers of skeletal muscle mass including lean mass and *vastus lateralis* muscle thickness. The magnitude of lean mass increase with HIIT was less than RET and must be interpreted with caution given this increase was similar to the CV of the densitometer. While all exercise training modalities increased muscle fiber size and lower body maximal strength, only HIIT and ENT increased aerobic capacity. After short-term detraining, lower body muscle strength gains were maintained, and lean mass gains persisted with RET and HIIT. In contrast, exercise training-induced increases in aerobic capacity with HIIT and ENT were not retained following detraining.

LINKING CHAPTERS 2 & 3

The results from the study described in Chapter 2 revealed that short-term divergent exercise modalities result in distinct skeletal muscle adaptations. First, all training groups increased lower body muscle strength. Firstly, all training groups increased lower body muscle strength. However, increases in upper body strength, lean mass and muscle thickness were exclusive to RET and HIIT. Second, type I and II muscle fiber CSA increased after exercise training independent of modality. Regarding detraining responses, lower body muscle strength was maintained in all training groups, however, declines in peak aerobic capacity were observed with HIIT and ENT. Additionally, lean mass was maintained with RET and HIIT after detraining. Taken together, these results show that short-term exercise training results in exercise-type dependent adaptation of skeletal muscle. Of note, HIIT increased several markers of indicative muscle growth and aerobic capacity whereas RET and ENT displayed more divergent responses.

There is a paucity of literature concerning molecular responses following short-term exercise training and detraining that underpin changes in muscle mass and size in human skeletal muscle. Accordingly, to better understand the molecular basis for these physiological responses, gene and protein expression analyses were performed on resting muscle samples obtained after exercise training and detraining (Chapter 3). Select targets were also used to associate alterations in basal levels of mRNA transcripts or protein content with exercise training- and detraining-induced changes in anabolic/catabolic skeletal adaptive responses.

CHAPTER 3

Experimental Chapter 2

Manuscript in preparation

3.1 ABSTRACT

Background: The expression and abundance of genes and proteins that mediate changes in skeletal muscle mass in middle-aged men remain poorly described. Accordingly, selected gene and protein targets recently shown to be implicated in exercise training-induced muscle growth (i.e., the androgen, apelin and vitamin D receptor) were measured before and after short-term single-mode exercise training and after detraining.

Methods: Thirty-five sedentary males (39 ± 3 y) performed six weeks of either ENT (n=12), HIIT (n=12) or RET (n=11) followed by 2.5 weeks of detraining. Resting muscle biopsies from the *vastus lateralis* were obtained at baseline, after exercise training and following detraining. Immunoblotting and targeted gene expression assays were performed to determine mRNA expression and total protein of selected anabolic and catabolic markers implicated in exercise training- and detraining. Pearson's correlations were performed between selected molecular markers and measurements of muscle mass/size.

Results: Exercise training-induced increases in androgen receptor protein, vitamin D receptor and apelin receptor mRNA independent of modality (all, $P < 0.05$). Exercise training led to similar increases in Akt and mTOR, but not p7S6K or 4E-BP1, protein expression (both, $P < 0.05$) independent of the mode of exercise training. Exercise training-induced increases in androgen receptor protein and vitamin D receptor mRNA were maintained after detraining. A significant positive correlation was observed following exercise training between the change in androgen receptor protein and leg lean mass in the HIIT group only ($P = 0.007$, $r = 0.751$)

Conclusion: These findings demonstrate that androgen receptor and vitamin D receptor, markers of muscle growth in human skeletal muscle, were upregulated in response to short-

term single-mode exercise training in middle-aged men. The post-exercise training increase in expression of androgen receptor and vitamin D receptor was not dependent on exercise mode. Moreover, expression of androgen receptor and vitamin D receptor were unaffected by a short period of detraining.

3.2 INTRODUCTION

Maintaining skeletal muscle mass through exercise training during middle adulthood is essential to counteract the natural loss of muscle protein and function with advancing age, commonly referred to as sarcopenia (Sayer *et al.*, 2008). In this regard, the mode of exercise performed is a key determinant of skeletal muscle phenotype and function. Resistance exercise training (RET) stimulates the activity of the IGF-Akt-mTOR pathway (Apró & Blomstrand, 2010; Camera *et al.*, 2010; Koopman *et al.*, 2006; Wilkinson *et al.*, 2008) to increase rates of muscle protein synthesis that when performed repeatedly lead to increases in muscle mass and strength (McGlory *et al.*, 2017a). In contrast, aerobic-based endurance exercise training (ENT) and high-intensity interval training (HIIT) both increase mitochondrial content and cardiorespiratory fitness (Gibala *et al.*, 2012), although accumulating evidence demonstrates that ENT can also increase muscle fiber size (Fry *et al.*, 2014; Harber *et al.*, 2009b; Harber *et al.*, 2012) and lean mass (Jonvik *et al.*, 2019; Knuiman *et al.*, 2019). However, considerably less is known about the regulation of key molecular transducers mediating skeletal muscle anabolism with ENT-based exercise. Moreover, when assessing the expression of genes and proteins implicated in the regulation of muscle growth, few studies have compared different exercise training modalities (Stefanetti *et al.*, 2015; Wilkinson *et al.*, 2008), particularly when considering short-term (4-8 weeks) interventions (de Souza *et al.*, 2013). Such information is important to provide a better understanding of whether the genes and proteins purported to mediate RET-induced muscle hypertrophy are similarly regulated by HIIT and ENT in human skeletal muscle, thereby offering the potential for other training options to mitigate losses of lean mass with aging. Recently, the androgen receptor (Mitchell *et al.*, 2013; Morton *et al.*, 2018), apelin receptor (Stokes *et al.*, 2020) and vitamin D receptor (Bass *et al.*, 2020) have been suggested as new candidates that contribute to RET-induced muscle growth. However, no study has directly compared the expression of these targets in response to HIIT and ENT.

In contrast to the multitude of studies that have investigated the expression of genes and proteins following exercise training, considerably less is known about the molecular responses with detraining. Detraining is the partial or complete loss of exercise training-induced adaptations due to a reduction or cessation in exercise frequency, intensity or duration (Mujika & Padilla, 2000). Defining the expression of genes and proteins after detraining provides insight into the molecular mechanisms that govern reductions in muscle mass and size. Accordingly, the purpose of this explorative study was to investigate the expression of selected genes and proteins established or recently purported to be implicated in mediating muscle growth following either short-term RET, ENT or HIIT, as well as in response to a period of detraining in sedentary, middle-aged men. A secondary aim was to measure markers regulating mitochondrial biogenesis and substrate metabolism exercise adaptation responses after short-term exercise training and detraining. As lean mass increased post-exercise training with RET and HIIT but not ENT (Chapter 2), it was hypothesised that the expression of genes and proteins implicated in muscle anabolism would follow a similar pattern.

3.3 METHODS

3.3.1 Participants & ethics approval

Thirty-five males (age 39 ± 3 y; body mass 94 ± 13 kg; body mass index [BMI] 29 ± 3 kg·m⁻²), who were not meeting current national physical activity guidelines participated in this study. Exclusion criteria included: age <35 or >45 y, BMI <25 or >35 kg·m⁻², smoking, type 2 diabetes mellitus, regular use of non-steroidal anti-inflammatory medication, scheduling conflicts prohibiting morning exercise session attendance and previous injuries exacerbated by exercise. The study was approved by the Australian Catholic University Human Research Ethics Committee (#2017-104H), prospectively registered online (ACTRN12617000894392; 19/06/2017) and conducted in accordance with the most recent revisions of the Declaration of Helsinki. The study was undertaken at the exercise physiology laboratories at the Australian Catholic University's St Patrick's campus (Fitzroy, VIC, Australia).

3.3.2 Study design and overview

Using a parallel groups design, participants in this study were randomly allocated to either RET (n=12), HIIT (n=12) or ENT (n=11) for six weeks of exercise training and 2.5 weeks of detraining (**Appendix A**). Measurements of aerobic capacity, muscle strength, body composition, muscle fiber characteristics from resting skeletal muscle biopsies, resting metabolic rate, glucose homeostasis and physical activity were taken at baseline and after exercise training and detraining as described in Chapter 2. Resting skeletal muscle biopsies were also used to perform gene and protein expression analyses (described below). Briefly, participants were provided a standardised meal (45% carbohydrate, 25% protein and 30% fat; 33% of total daily energy intake (~3700-4300 kJ) based on the Cunningham equation (Cunningham, 1980) for consumption the evening prior to a muscle biopsy. Participants reported to the laboratory in an overnight fasted state (~10 h) between 0630-0730 h on the day of a muscle biopsy. A resting percutaneous muscle biopsy from the *vastus lateralis* (~100-200

mg) was obtained, under local anaesthesia (2–3 mL of 1% Xylocaine) using a Bergström needle (Bergstrom, 1975) modified for manual suction. The sample was frozen in liquid nitrogen and stored at -80 °C until analysis. The post-exercise training muscle biopsy was obtained ~48 h following the final exercise session. The DT muscle biopsy was obtained at rest.

3.3.3 Exercise training protocols

The exercise training and detraining interventions are described in detail in Chapter 2. Briefly, participants trained on three non-consecutive mornings per week and progressive overload was applied to all exercise training protocols. Every session for each group commenced with a short warm-up (three minutes low-intensity cycling) and finished with a cool-down (three minutes low-intensity cycling). The RET group performed upper and lower body weight-bearing exercise using pulley machines and free weights. Sets ranged from 3-4 and repetitions from 9-12 at 60-80% one repetition maximum (1RM), with a standardised 3 min of rest between sets for all exercise movements. The HIIT group performed 13-23 min of stationary cycling that consisted of 30-60 s work periods at 90-130% PPO with one minute of recovery at a power output of 50 W. The number of repetitions varied from 8-15 depending on the work period duration for that session. The ENT group performed continuous stationary cycling at submaximal intensities for 30-52 min at 50-75% PPO.

3.3.4 Immunoblotting

Skeletal muscle (~25 mg) was homogenised in lysis buffering containing 50 mmol·L⁻¹ Tris-HCl (pH 7.5), 1 mmol·L⁻¹ EDTA, 1 mmol·L⁻¹ EGTA, 10% glycerol, 1% Triton X-100, 50 mmol·L⁻¹ sodium fluoride, 5 mmol·L⁻¹ sodium pyrophosphate, with complete protease inhibitor and PhosSTOP phosphatase inhibitor cocktail tablets (Sigma-Aldrich, St. Louise, MO, USA). Protein quantification was undertaken using a bicinchoninic acid assay (Pierce BCA Protein Assay Kit, ThermoFisher Scientific) with all samples measured in triplicate. Samples were

diluted to $2\ \mu\text{g}\cdot\mu\text{L}^{-1}$ and $10\ \mu\text{g}$ was run for each sample on 4-20% pre-cast stainfree gels (Bio-Rad, Hercules, CA, USA) and separated by SDS-PAGE prior to transfer to PVDF membranes (Merck Millipore, Burlington, MA, USA). Membranes were blocked in 7.5% bovine serum albumin (BSA) diluted in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) for 1 hr at room temperature, and then incubated overnight at $4\ ^\circ\text{C}$ in commercially available primary antibodies for androgen receptor (1:1000, #3202, Cell Signaling Technology), Akt (1:1000, #4691, Cell Signaling Technology), mammalian target of rapamycin (mTOR; 1:1000, #2972, Cell Signaling Technology), ribosomal protein S6 kinase beta-1 (p70S6K; 1:1000, #9202, Cell Signaling Technology), eukaryotic translation initiation factor 4E (4E-BP1; 1:1000, #9644, Cell Signaling Technology), citrate synthase (1:1000, ab96600, abcam), hexokinase II (1:1000, #2867, Cell Signaling Technology) and carnitine palmitoyltransferase (CPT1b; 1:1000, ab134988, abcam). Membranes were washed in TBS-T ($3 \times 5\ \text{min}$) and incubated in the corresponding secondary antibody as specified by the supplier. Membranes were detected via enhanced chemiluminescence detection using SuperSignal West Femto Maximum Sensitivity Substrate (Life Technologies, Carlsbad, CA, USA) and Bio-Rad ChemiDoc MP Imaging System (Hercules, CA, USA). A pooled sample of muscle lysate was run on every gel as an internal control. The volume density of each target band was quantified using Bio-Rad Image Lab 6.0.1 and normalised to the total protein of the control sample using stain-free imaging technology (Bio-Rad). In this study, 35 participants (ENT: n=12, HIIT: n=12, RET: n=11) were included for protein expression analysis. However, due to a lack of muscle tissue, a small number of samples could not be included at specific time points within each group. As such, 11 participants were included for analysis at Pre in HIIT, 10 participants at Pre in RET, 10 participants at Post in ENT and 10 participants at DT in RET.

3.3.5 RNA extraction, quantification, reverse transcription and real-time PCR

A TrIzol-based kit (Thermo Fisher Scientific) was used to extract RNA from skeletal muscle tissue. Skeletal muscle (20 mg) was homogenised in TRIzol with chloroform to form an aqueous RNA phase. The RNA phase was then mixed with isopropanol alcohol to form a RNA precipitate. Subsequently, the resulting pellet was washed and resuspended in 40 µL of RNase-free water. A spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific) was used to quantify extracted RNA. Reverse transcription and real-time PCR was performed as previously described (Camera *et al.*, 2015). First-strand cDNA synthesis was performed using TaqMan Reverse Transcription Reagents (Thermo Fisher Scientific). Quantification of mRNA (in duplicate) was performed by using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). TaqMan FAM-labelled primer/probes for apelin receptor (*APLNR*; Hs00270873_s1), F-box only protein 32 (*FBXO32*; Hs01041408_m1; described as atrogin-1), myogenic differentiation 1 (*MYOD1*; Hs00159528_m1), myogenin, (*MYOG*; Hs01072232_m1), myostatin (*MSTN*; Hs00976237_m1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*; Hs00173304_m1; described herein PGC1-α), tripartite motif containing 63 (*TRIM63*; Hs01041408_m1; described as muscle-RING finger protein-1 (MuRF-1)), vascular endothelial growth factor A (*VEGFA*; Hs00900055_m1) and vitamin D receptor (*VDR*; Hs01045843_m1) were used in a final reaction volume of 20 µL. Target mRNA expression relative to the geometric mean of three references genes, 18S ribosomal RNA (*18S*; Hs99999901_s1), beta-2-microglobulin (*B2M*; Hs00187842_m1) and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; Hs02786624_g1), was calculated using the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen, 2001) and expressed as arbitrary units (AU). mRNA expression cycle threshold values of the geometric mean of reference genes were stably expressed across all time points and between different exercise conditions (data not shown).

3.3.6 Statistical analyses

Data normality was assessed prior to statistical analyses by assessing skewness, kurtosis and results from Shapiro-Wilk tests. Linear mixed effect models (LME), with subject for random intercept, were used to determine main effects of fixed factors: *time* (Pre, Post, DT), *group* (ENT, HIIT, RET), and interaction (*time* $\times$ *group*) from which residuals were plotted on a histogram to inspect data distribution. Where significant main effects were observed (i.e., *time* or *group*) post hoc comparison with Bonferroni correction was used to locate differences. When significant interaction effects (*time* $\times$ *group*) were observed, post hoc comparison with Bonferroni corrections was used to determine within and/or between group differences. Pearson's correlations were performed between the post-exercise training changes (delta) in gene (apelin receptor, vitamin D receptor) or protein (androgen receptor) expression and the post-exercise training change (delta) in muscle fiber size (type I, type II) or lean mass (total, leg). Significance was accepted at $P < 0.05$. All data are presented as mean $\pm$ standard deviation.

3.4 RESULTS

3.4.1 Protein expression

Androgen receptor/Akt/mTOR/p70S6K/4E-BP1

At baseline, there were no significant differences in androgen receptor total protein expression between groups (**Figure 3.1**). A significant main effect of *time* ($P=0.021$) was observed for androgen receptor total protein expression (**Figure 3.1A**). Post-hoc comparisons showed that androgen receptor total protein expression increased significantly after exercise training in all groups ($P=0.048$). After detraining, androgen receptor total protein expression remained elevated compared to baseline in all groups ($P=0.045$). There were no significant differences in androgen receptor total protein expression between groups after exercise training or detraining. At baseline, there were no significant differences in Akt total protein expression between groups. A significant main effect of *time* ($P<0.001$) was observed for Akt total protein expression (**Figure 3.1B**). Post-hoc comparisons showed that Akt total protein expression increased significantly after exercise training in all groups ($P<0.001$). After detraining, Akt total protein expression decreased below post-exercise training levels ($P=0.001$) but remained elevated above baseline ($P<0.001$). There were no significant differences in Akt total protein expression between groups after exercise training or detraining.

At baseline, there were no significant differences in mTOR total protein expression between groups. A significant *time* $\times$ *group* interaction effect ($P=0.032$) was observed for mTOR total protein expression (**Figure 3.1C**). After exercise training, mTOR total protein expression increased significantly in all groups ($P<0.01$). After detraining, mTOR total protein expression decreased significantly compared to post-exercise training in the RET group ($P=0.001$) but remained elevated above baseline in the HIIT and ENT groups (both, $P<0.01$). There were no significant differences in mTOR total protein expression between groups after exercise training or detraining. At baseline, there were no significant differences in p70S6K total protein expression between groups. p70S6K total protein expression did not change after

exercise training or detraining in all groups (**Figure 3.1D**). At baseline, there were no significant differences in 4E-BP1 total protein expression between groups. A significant main effect of *time* ($P=0.026$) was observed for 4E-BP1 total protein expression (**Figure 3.1E**). Post-hoc comparisons showed that 4E-BP1 total protein expression increased significantly after detraining compared to baseline in all groups ($P=0.023$). There were no significant differences in 4E-BP1 total protein expression between groups after exercise training or detraining.

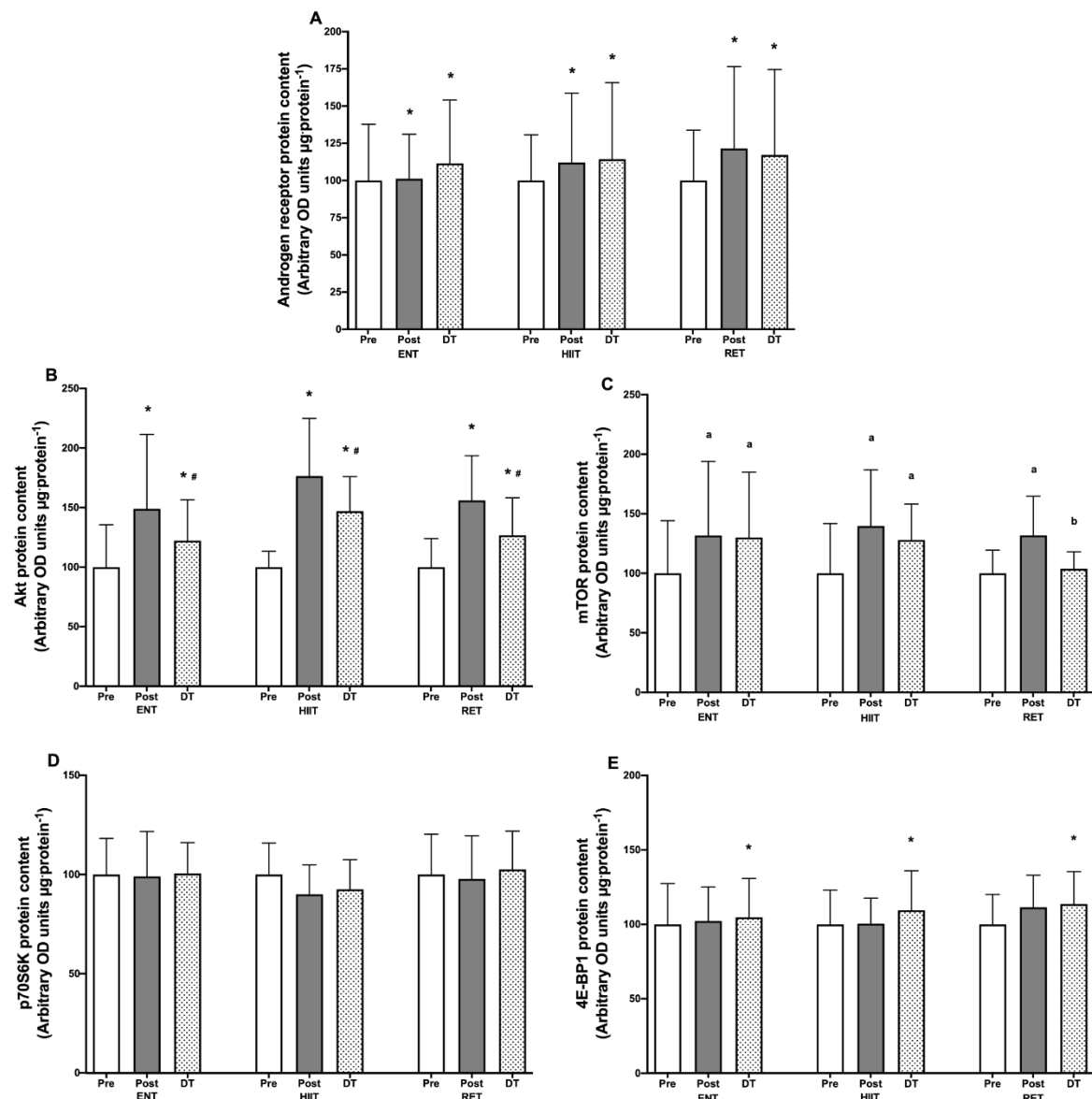


Figure 3.1 Total protein levels at baseline (Pre) of androgen receptor (A), Akt (B), mTOR (C), p70S6K (D) and 4E-BP1 (E) and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and standard deviation. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. a, $P<0.05$ vs Pre within group; b, $P<0.05$ vs Post within group; *, $P<0.05$ vs Pre (main effect of time); #, $P<0.05$ vs Post (main effect of time).

Citrate synthase/Hexokinase II/CPT1b

At baseline, there were no significant differences in citrate synthase total protein expression between groups (**Figure 3.2**). A trend for a main effect of *time* ($P=0.088$) was observed for citrate synthase total protein expression (**Figure 3.2A**). There were no significant differences in citrate synthase total protein expression between groups after exercise training or detraining. At baseline, there were no significant differences in hexokinase II total protein expression between groups. A significant main effect of *time* ($P<0.001$) was observed for hexokinase II total protein expression (**Figure 3.2B**). Post-hoc comparisons showed that hexokinase II total protein expression increased significantly after exercise training in all groups ($P<0.001$). After detraining, hexokinase II total protein expression decreased significantly compared to post-exercise training in all groups ($P<0.001$). There were no significant differences in hexokinase II total protein expression between groups after exercise training or detraining. At baseline, there were no significant differences in CPT1b total protein expression between groups. CPT1b total protein expression did not change after exercise training or detraining in all groups (**Figure 3.2C**).

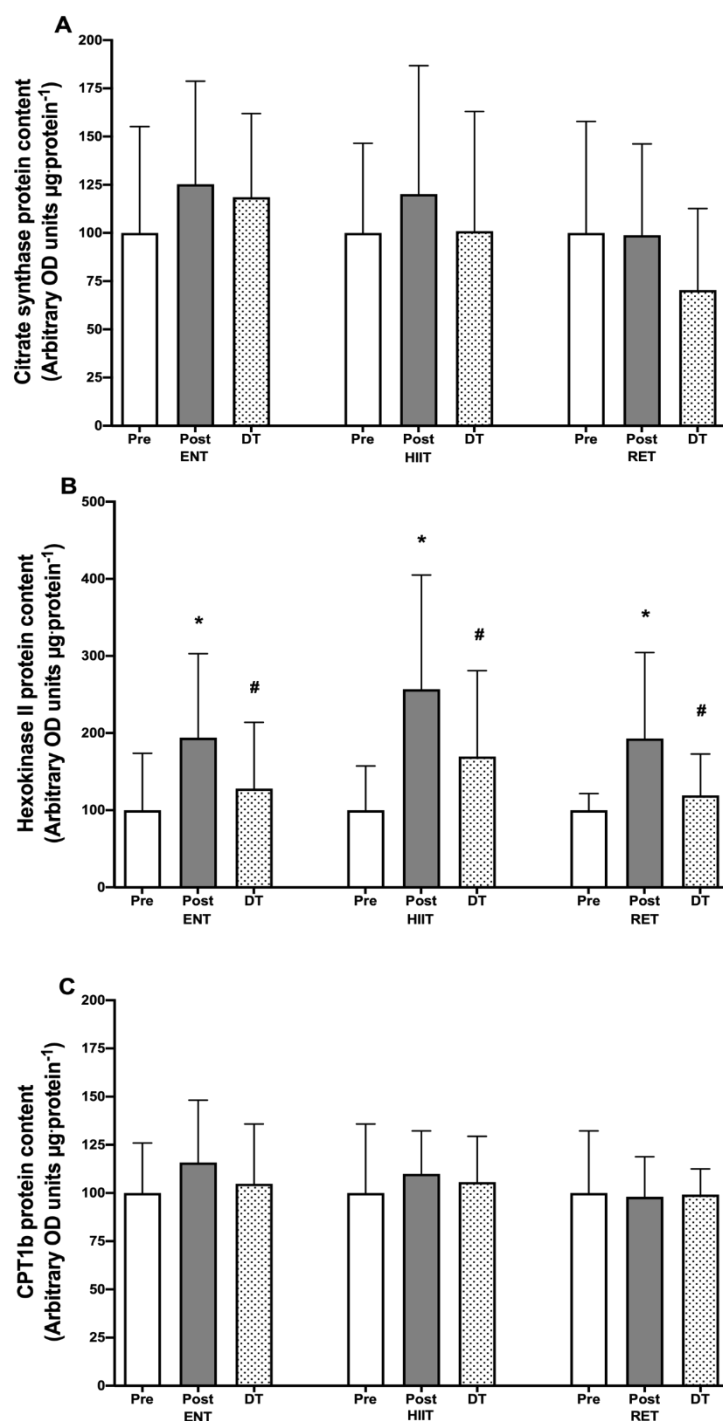


Figure 3.2 Total protein levels at baseline (Pre) of citrate synthase (A), hexokinase II (B) and CPT1b (C) and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and standard deviation. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. *, $P < 0.05$ vs Pre (main effect of time); #, $P < 0.05$ vs Post (main effect of time).

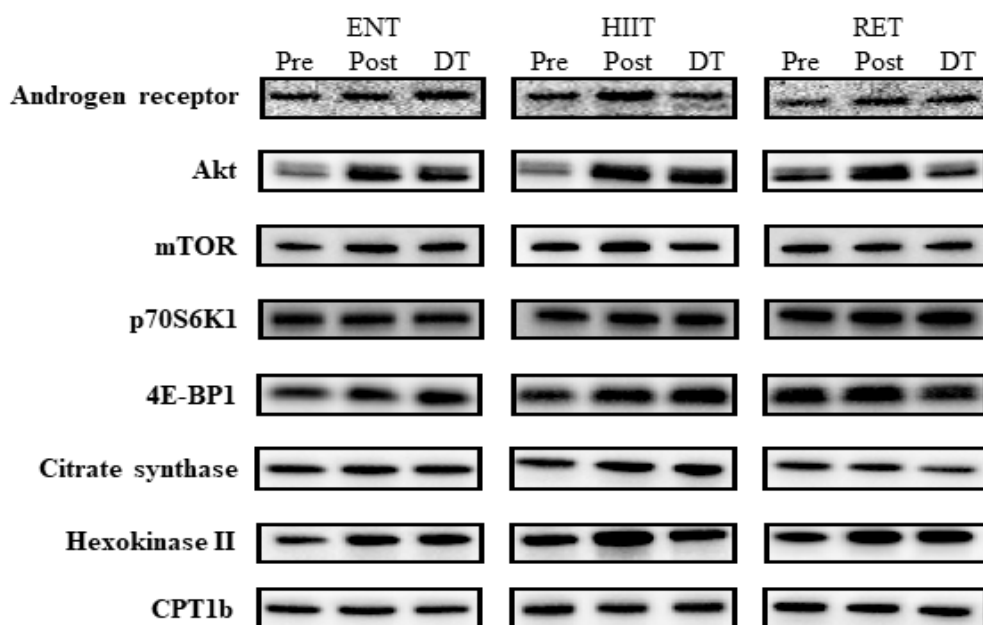


Figure 3.3 Representative protein blots at baseline (Pre) and following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

3.4.2 mRNA expression

Apelin receptor/Vitamin D Receptor/MYOD1/Myogenin

At baseline, there were no significant differences in apelin receptor mRNA expression between groups (**Figure 3.4**). A significant main effect of *time* ($P<0.001$) and *condition* ($P=0.036$) was observed for apelin receptor mRNA expression (**Figure 3.4A**). After exercise training, apelin receptor mRNA expression increased significantly compared to baseline in all groups ($P<0.001$). After detraining, apelin receptor mRNA expression decreased significantly compared to post-exercise training in all groups ($P=0.002$). Apelin receptor mRNA expression in the RET group was significantly greater than in the HIIT group ($P=0.039$). At baseline, there were no significant differences in VDR mRNA expression between groups. A significant main effect of *time* ($P<0.001$) was observed for VDR mRNA expression (**Figure 3.4B**). After exercise training, VDR mRNA expression increased significantly compared to baseline in all groups ($P=0.001$). After detraining, VDR mRNA expression remained elevated compared to baseline in all groups ($P=0.001$). There were no significant differences in VDR mRNA

expression between groups after exercise training or detraining. At baseline, there were no significant differences in MYOD1 mRNA expression between groups. A significant main effect of *condition* ($P<0.001$) was observed for MYOD1 mRNA expression (**Figure 3.4C**). MYOD1 mRNA expression in the ENT group was significantly greater than in the HIIT group ($P=0.001$) and RET group ($P=0.015$). At baseline, there were no significant differences in myogenin mRNA expression between groups. A significant main effect of *time* ($P<0.001$) was observed for myogenin mRNA expression (**Figure 3.4D**). After exercise training, there was a trend for an increase in myogenin mRNA expression compared to baseline in all groups ($P=0.096$). After detraining, there was a trend for an increase in myogenin mRNA expression compared to baseline in all groups ($P=0.058$). A trend for a main effect of *condition* ($P=0.058$) was observed for myogenin mRNA expression.

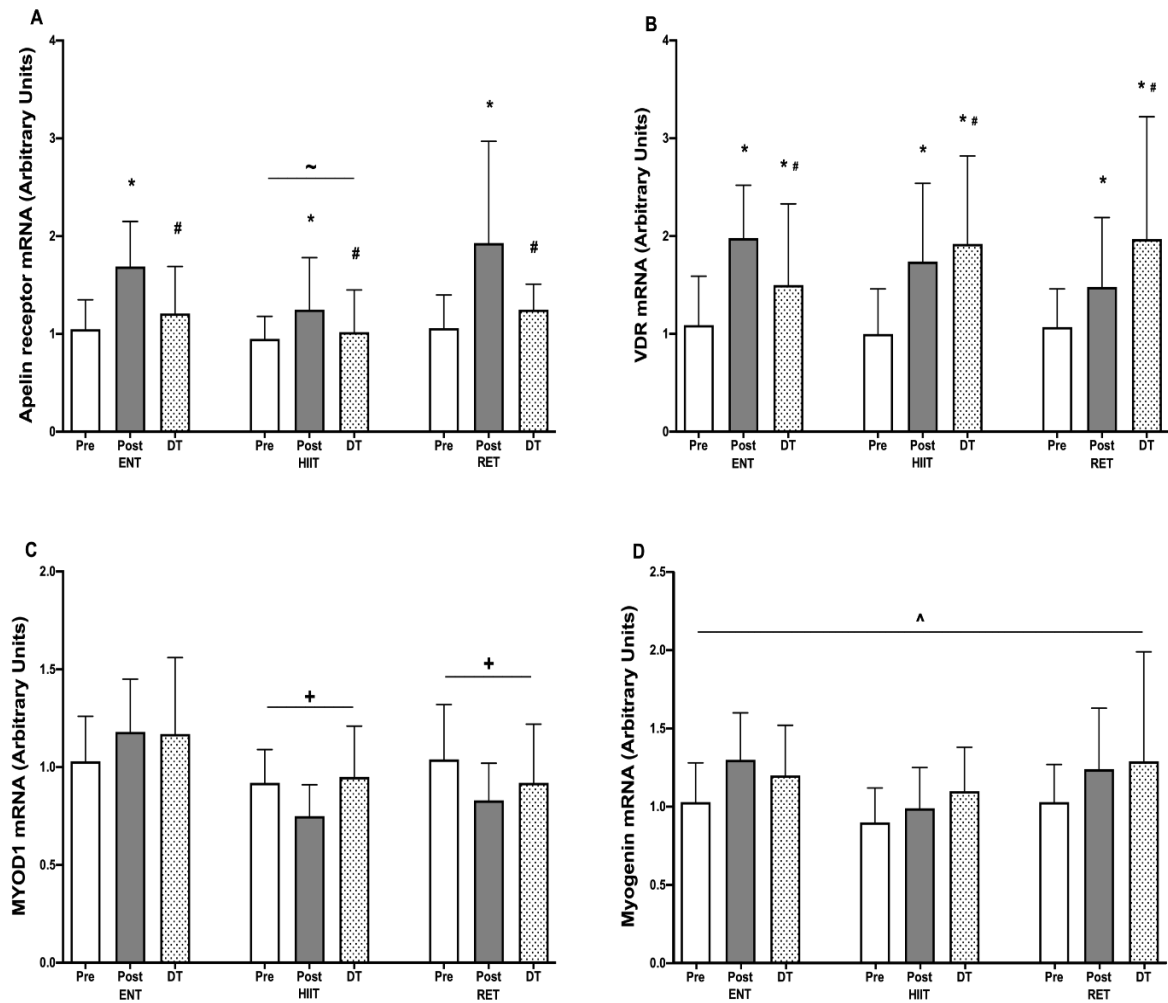


Figure 3.4 Baseline (Pre) apelin receptor (A), vitamin D receptor (B), MYOD1 (C) and myogenin (D) mRNA expression and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and standard deviation. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. *, $P < 0.05$ vs Pre (main effect of time); #, $P < 0.05$ vs Post (main effect of time); ^, overall main effect of time ($P < 0.05$) but no significant post-hoc comparisons; ~, main effect of condition ($P < 0.05$; RET significantly greater than HIIT); +, main effect of condition ($P < 0.05$; ENT significantly greater than HIIT and ENT).

MuRF-1/Atrogin-1/Myostatin

At baseline, there were no significant differences in MuRF-1 mRNA expression between groups (**Figure 3.5**). A significant main effect of *time* ($P=0.039$) and *condition* ($P=0.001$) was observed for MuRF-1 mRNA expression (**Figure 3.5A**). After detraining, MuRF-1 mRNA expression increased significantly compared to post-exercise training in all groups ($P=0.039$). MuRF-1 mRNA expression in the ENT group was significantly greater than in the HIIT group ($P=0.001$). At baseline, there were no significant differences in atrogin-1 mRNA expression between groups. A significant main effect of *condition* ($P<0.001$) was observed for atrogin-1 mRNA expression (**Figure 3.5B**). Atrogin-1 mRNA expression in the ENT group was significantly greater than in the HIIT group ($P=0.025$). At baseline, there were no significant differences in myostatin mRNA expression between groups. A significant main effect of *time* ($P<0.001$) was observed for myostatin mRNA expression (**Figure 3.5D**). After exercise training, myostatin mRNA expression decreased significantly compared to baseline in all groups ($P<0.001$). After detraining, myostatin mRNA expression increased significantly compared to post-exercise training in all groups ($P=0.001$). There were no significant differences in myostatin mRNA expression between groups after exercise training or detraining.

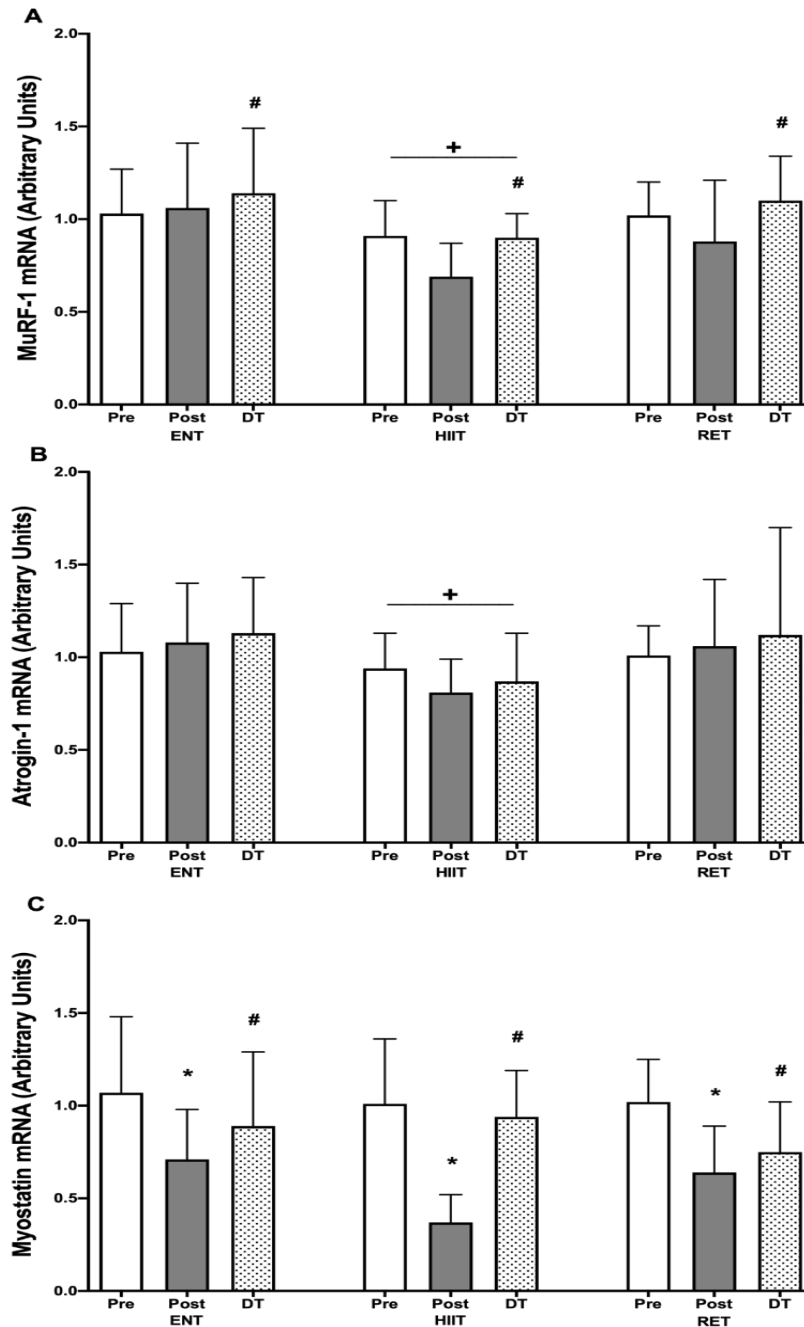


Figure 3.5 Baseline (Pre) MuRF-1 (A), atrogin-1 (B) and myostatin (C) mRNA expression and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and standard deviation. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. *, $P < 0.05$ vs Pre (main effect of time); #, $P < 0.05$ vs Post (main effect of time); +, main effect of condition ($P < 0.05$; ENT significantly greater than HIIT).

PGC-1 α /VEGF/NR4A3

At baseline, there were no significant differences in PGC-1 α mRNA expression between groups (**Figure 3.6**). A significant main effect of *condition* ($P<0.001$) was observed for PGC-1 α mRNA expression (**Figure 3.6A**). PGC-1 α mRNA expression in the ENT group was significantly greater than in the HIIT group ($P<0.001$) and RET group ($P=0.035$). A trend for a main effect of *time* ($P=0.058$) was observed for PGC-1 α mRNA expression. At baseline, there were no significant differences in VEGF mRNA expression between groups. A significant main effect of *condition* ($P<0.001$) was observed for VEGF mRNA expression (**Figure 3.6B**). VEGF mRNA expression in the ENT group was significantly greater than in the HIIT group ($P<0.001$). At baseline, there were no significant differences in NR4A3 mRNA expression between groups. A significant main effect of *condition* ($P<0.001$) was observed for NR4A3 mRNA expression (**Figure 3.6C**). NR4A3 mRNA expression in the RET group was significantly greater than in the HIIT group ($P=0.002$).

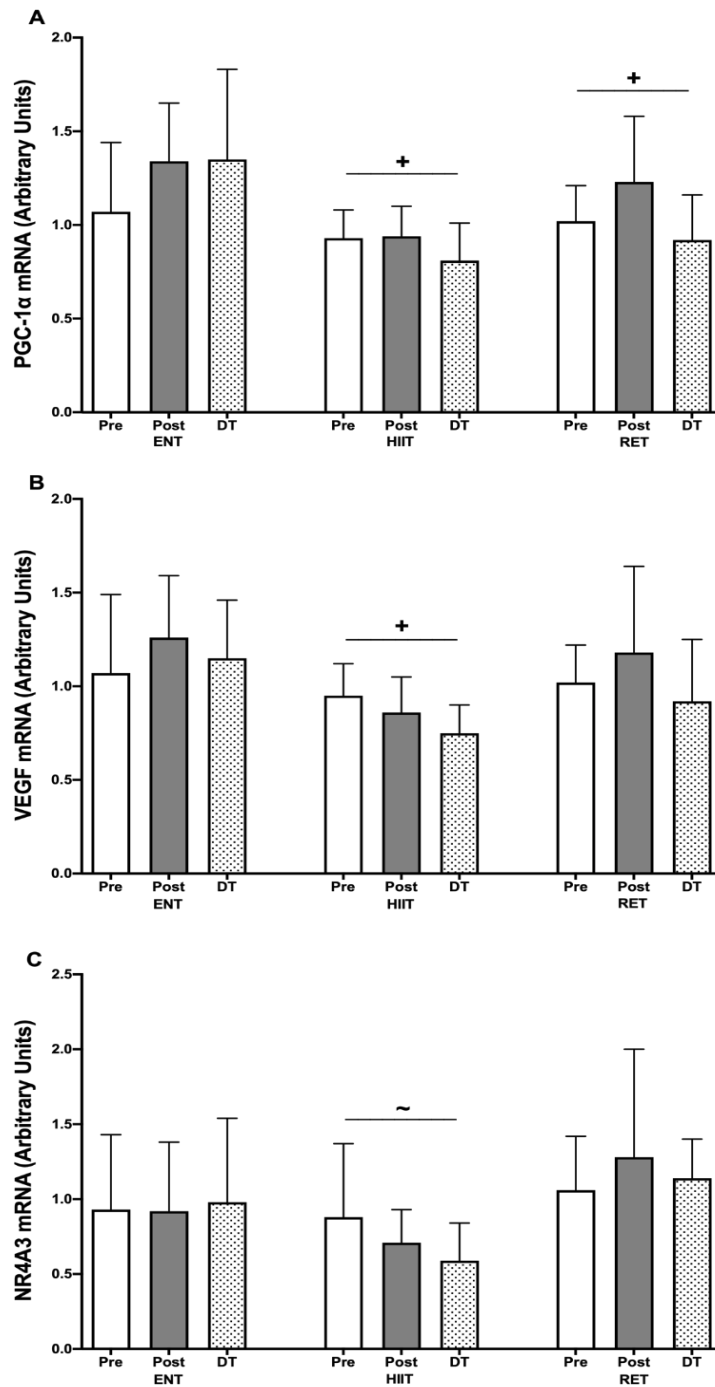


Figure 3.6 Baseline (Pre) PGC-1 α (A), VEGF (B) and NR4A3 (C) mRNA expression and changes following six weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

Data are presented as mean and standard deviation. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. +, main effect of condition ($P < 0.05$; ENT significantly greater than HIIT); ~, main effect of condition ($P < 0.05$; RET significantly greater than HIIT).

3.4.3 Correlations between Gene or Protein Markers of Muscle Anabolism and Physiological Outcomes

There was a significant positive relationship between the change in androgen receptor protein expression and change in leg lean mass from baseline to post-exercise training in the HIIT group ($r=0.751$, $P=0.007$). In contrast, no significant correlations were observed between change in androgen receptor protein expression and change in leg lean mass from baseline to post-exercise training with RET and ENT. There were no significant relationships between post-exercise training changes in gene (apelin receptor, vitamin D receptor) or protein (androgen receptor) expression and the post-exercise training change in muscle fiber size (type I or type II) or lean mass (total or leg).

3.5 DISCUSSION

Acute and transient changes in gene transcription following exercise-induced contraction repeated over weeks and months ultimately form the basis of skeletal muscle training adaptation and subsequent improvements in exercise capacity (Perry *et al.*, 2010). In light of observed changes in post-exercise training and detraining lean mass, muscle strength and VO_{2max} observed in the investigation described in Chapter 2, the aim of the current study was to determine the expression and association of both established and selected novel gene and protein targets that may underpin a molecular basis to help explain some of these responses. The findings reveal that exercise training-induced changes in androgen receptor protein content, vitamin D receptor and apelin receptor mRNA are all independent of exercise training modality. Of note was that the exercise training-induced increases in androgen receptor protein and vitamin D receptor mRNA were maintained after a short period of detraining suggesting both these markers may, in part, have contributed to the maintenance of lean mass during this period of exercise cessation described in Chapter 2.

The first major finding of this study was that short-term (six weeks) of exercise training resulted in an increased protein expression of the skeletal muscle androgen receptor, independent of training modality. Uniform increases in skeletal muscle androgen receptor protein expression were somewhat unexpected when considering RET and HIIT resulted in ~three- and ~two-fold greater increases in total lean mass, respectively, than ENT (Chapter 2). The binding of androgens to the nuclear androgen receptor in skeletal muscle cause its translocation to the nucleus where, upon binding to specific DNA sequences, it promotes the transcription of several anabolic gene targets that may promote muscle hypertrophy (McCullough *et al.*, 2020; Wilkenfeld *et al.*, 2018). No previous study has reported increases in androgen receptor protein content in human skeletal muscle following short-term HIIT or ENT. Indeed, most studies in humans have only assessed skeletal muscle androgen receptor mRNA or protein expression following several months of RET. While some studies report an

increase in androgen receptor mRNA (Haun *et al.*, 2018) or protein expression (Sato *et al.*, 2014), others have observed no change (Ahtiainen *et al.*, 2011; Mitchell *et al.*, 2013; Morton *et al.*, 2018; Nilsen *et al.*, 2016). Nonetheless, increases in intramuscular androgen receptor content have been associated with prolonged RET-induced muscle hypertrophy (Ahtiainen *et al.*, 2011; Mitchell *et al.*, 2013; Morton *et al.*, 2018) although such a finding is not universal (Mobley *et al.*, 2018).

Limited information exists regarding androgen receptor expression following short periods (i.e., six weeks) of strength-based training. Haun *et al.* (2019a) reported no change in androgen receptor protein content in well-trained young men following six weeks of RET, while Gharahdaghi *et al.* (2019) observed increased androgen receptor mRNA in healthy older (65-75 years) men after six weeks of RET, but only with adjuvant testosterone therapy. No such pharmaceutical therapy was administered in the current study, however participants were instructed to ingest ~ 1.4 g·kg BW⁻¹ of dietary protein throughout the exercise training to provide nutritional support for promoting lean mass accrual. Specifically, ~ 30 g of whey protein was ingested after every exercise session and an eating plan was prescribed to encourage even distribution of daily protein intake across main meals to maximise daily rates of MPS (McKendry *et al.*, 2020). Amino acids from dietary protein, particularly leucine, are known to activate the mTORC1 signalling cascade (De Bandt, 2016). However, in the absence of a group that did not receive supplemental protein, it cannot be definitively concluded that increased protein availability contributed to increased androgen receptor protein content. When considering findings from the current study and those from Gharahdaghi *et al.* (2019), it is likely that middle-aged and older adults may benefit from adjunct nutritional and/or therapeutic strategies with short-term RET to promote increases in androgen receptor content which appears to be a potent molecular transducer driving muscle hypertrophy (Clarke *et al.*, 2019; Wu *et al.*, 2017; Yin *et al.*, 2020).

Given recent evidence implicating associations between androgen receptor expression and RET-induced muscle hypertrophy (Mitchell *et al.*, 2013; Morton *et al.*, 2018), correlations were undertaken between the changes in anabolic genes (apelin and vitamin D receptor)/proteins (androgen receptor) and changes in measurements of muscle growth (muscle fiber CSA and lean mass) after different types of exercise training. Indeed, this is the first study to correlate changes in androgen receptor protein expression with indices of muscle hypertrophy following HIIT and ENT. Within-group correlations were conducted to identify exercise-specific responses between anabolic proteins and markers of muscle growth. A significant correlation between change in androgen receptor protein and leg lean mass was observed after exercise training with HIIT only. While a single bout of HIIT does not change circulating testosterone levels in young men (Velasco-Orjuela *et al.*, 2018), six weeks of HIIT has been shown to increase free testosterone in sedentary older men (Hayes *et al.*, 2017) and masters athletes (Herbert *et al.*, 2017). While the current study did not measure circulating levels of testosterone, it might be speculated that levels of free testosterone may similarly have increased post-exercise training as a response to enhance androgen receptor genesis in skeletal muscle and contribute to the correlated increases in leg lean mass observed following HIIT.

When considering the relationship between changes in muscle fiber CSA and androgen receptor protein expression, the current study is consistent with one other that reported no association after 12 weeks of RET in young untrained men (Haun *et al.*, 2018). In contrast, other studies have reported a positive correlation between muscle fiber CSA and androgen receptor protein expression following prolonged RET in young untrained (Mitchell *et al.*, 2013), resistance-trained (Morton *et al.*, 2018) and older men (Ahtiainen *et al.*, 2011). Discrepant findings may be explained by differences in training duration (six vs ≥ 12 weeks), baseline fitness (sedentary vs resistance-trained) and age of participants (young vs old). Another consideration that may, in part, explain the equivocal results above is the research question and

subsequent decisions informing allocation to exercise groups (e.g., comparing high and low responders (Morton *et al.*, 2018) vs protein supplementation sources (Haun *et al.*, 2018)).

Similar to exercise-induced changes in total protein levels of androgen receptor, mRNA levels of vitamin D receptor also increased independent of exercise modality. A meta-analysis of 30 randomised controlled trials demonstrated vitamin D supplementation can induce a significant positive effect on muscle strength in older adults (Beaudart *et al.*, 2014), indicating a potential skeletal muscle anabolic response. However, there is a paucity of data from human studies assessing vitamin D receptor expression in response to exercise. Recently, vitamin D receptor mRNA expression was associated with a greater increase in lean mass following 20 weeks of RET in human skeletal muscle of adults (aged 18-75 y) (Bass *et al.*, 2020). In agreement with results from that study (Bass *et al.*, 2020), an increase in vitamin D receptor mRNA was observed in the current study after short-term resistance exercise training in middle-aged men and, for the first time, following cycling HIIT and ENT. One of the mechanisms by which increased vitamin D receptor mRNA expression may contribute to skeletal muscle anabolism is via the activation of the IGF-1-Akt-mTOR pathway (Dzik & Kaczor, 2019). While activation of this pathway was not measured due to the timing of skeletal muscle biopsies, total Akt and mTOR protein content increased with all training modes post-exercise training. In contrast, there were no changes in total protein content of the downstream targets p70S6K and 4E-BP1. Previous work has demonstrated eight weeks of RET, but not HIIT, increased Akt and p70S6K1 total protein content in untrained young men (de Souza *et al.*, 2013).

The current study is the first to compare the expression of the mTOR signalling pathway regulating translation initiation responses between RET, HIIT and ENT in human skeletal muscle. The similar magnitudes of observed increases in total mTOR and Akt protein expression between the three exercise modes following short-term exercise training follows a similar pattern to the acute changes in phosphorylation of mTOR pathway proteins between different exercise modes (Camera *et al.*, 2010); Wilkinson *et al.* (2008). As such, these

observations may support the notion that mTORC1-mediated alterations in translation initiation do not significantly contribute to the ‘specificity of training adaptation’, particularly anabolic-related responses, in skeletal muscle with diverse contractile modes. However, it must be acknowledged that acute and transient changes in protein phosphorylation do not necessarily reflect changes in total protein content.

Another finding from the current study was that short-term exercise training-induced increases in the expression of skeletal muscle apelin receptor mRNA following independent of exercise modality. Traditionally considered an ‘adipokine’ (Carpéné *et al.*, 2007), the peptide apelin has been shown to be upregulated following exercise training in obese young men (Besse-Patin *et al.*, 2014) and older adults (Bae *et al.*, 2019). Moreover, circulating levels of apelin were demonstrated to be positively associated with enhanced physical function (i.e., improved test results for short physical performance battery) in older adults (Vinel *et al.*, 2018). Nonetheless, the role of apelin, or its receptor, in promoting human skeletal muscle function, remains largely unknown. Work in muscle fibers taken from young and older adults show acute apelin treatment increases Akt, mTOR and p70S6K phosphorylation while also reducing FOXO-3 and 4E-BP1 phosphorylation in older muscle fibers only (Vinel *et al.*, 2018). Additionally, apelin supplementation reduced MuRF-1 and atrogin-1 mRNA expression in middle-aged and older mice (Vinel *et al.*, 2018), suggesting apelin treatment can promote protein synthesis and suppress proteolysis. Following 10 weeks of RET in recreationally active young men, Stokes *et al.* (2020) reported increased gene expression of the apelin receptor which was identified as a positive mediator of muscle growth. The results of the current study confirm this previous finding by showing that only six weeks of RET in middle-aged men increases apelin receptor mRNA. However, as no correlation was observed between apelin gene abundance and changes in lean mass with RET, the degree to which apelin mediates muscle growth responses with RET requires further interrogation. It was also found that short-term HIIT and ENT can increase apelin receptor mRNA. Besse-Patin *et al.* (2014) observed increases

in apelin mRNA, but not apelin receptor, following eight weeks of submaximal cycling and running exercise in sedentary obese young men. The disparity with the current study findings may relate to differences in population age (young vs. middle-aged men) and post-exercise training biopsy sampling time point (~72 vs 48 h). The current work is the first to investigate and report increased apelin receptor mRNA following HIIT in human skeletal muscle. Further work elucidating both the mechanistic role and time course of apelin and its receptor in exercise adaptation responses following HIIT are warranted to better understand the degree to which apelin may mediate increases in muscle growth following exercise.

When examining the effect of a short-term period of detraining on cellular responses, exercise training-induced increases in androgen receptor protein content and vitamin D receptor mRNA, but not apelin receptor mRNA, were maintained after exercise training cessation. No previous study has assessed gene or protein expression of these molecular markers implicated in muscle growth following a period of exercise training cessation in human skeletal muscle. Indeed, there is a clear knowledge gap regarding changes to molecular transducers that are linked to processes governing purported reductions in muscle mass in response to ceasing exercise training in human skeletal muscle. In the present study, detraining selectively downregulated mTOR protein content induced by RET but not HIIT or ENT. Leger *et al.* (2006) reported mTOR²⁴⁴⁸ phosphorylation was unchanged after eight weeks of detraining that preceded eight weeks of RET. Considering the transient nature of protein phosphorylation events, it is possible this result may be more indicative of a resting (i.e., unstimulated) response.

Regarding changes in mRNA levels of surrogate markers of muscle protein breakdown/proteolysis, the short-term period of detraining significantly increased myostatin and MuRF-1 mRNA compared to post-exercise training levels, independent of exercise modality. Previous work has demonstrated eight weeks of detraining reduced training-induced increases in MuRF-1 and atrogin-1 mRNA abundance to baseline levels (Leger *et al.* (2006). It is likely the longer detraining period between the previous and current work may help explain

this disparity in findings. Regardless, despite the observed increases in MuRF-1 and myostatin gene expression, exercise-training induced increases in muscle hypertrophy and strength were retained following detraining. The maintenance of such physiological adaptations may indicate the detraining period was insufficient in duration or level of unloading (i.e., participants performed activities of daily living but did not exercise) for such catabolic markers to significantly impact muscle morphology and function. Additionally, it is also plausible the increases in these catabolic markers may be more representative of enhanced muscle remodelling processes to remove damaged contractile proteins induced during the exercise training intervention, particularly considering the sedentary nature of the participant cohort.

Finally, expression of several genes and proteins implicated in substrate metabolism and mitochondrial biogenesis responses to exercise were also measured. While six weeks of exercise training increased protein content of hexokinase II irrespective of exercise modality, protein levels of citrate synthase and CPT1b protein as well as PGC1 α and VEGF mRNA were unchanged after both exercise training and detraining. A recent systematic literature review reported changes in PGC-1 α expression were capable of significantly enhancing aerobic performance in mice (Yaghoob Nezhad *et al.*, 2019). Even though significant increases in VO_{2max} were observed post-intervention with HIIT and ENT, the short-term nature of the training intervention may have been inadequate to significantly increase basal transcript levels of PGC-1 α (Granata *et al.*, 2018). Moreover, mRNA levels of NR4A3, a recently identified nuclear receptor mediating exercise-induced metabolic responses and demonstrated to be responsive to both exercise and inactivity stimuli (Pillon *et al.*, 2020), was similarly unchanged after exercise training and detraining. Future studies in human skeletal muscle elucidating the impacts of exercise volume, intensity and duration are needed to better characterise sensitivity of NR4A3 to divergent contractile stimuli.

There are several limitations to the current study which should be acknowledged. Firstly, this study was conducted only in men and thus cannot rule out that alterations in gene

and protein expression may be impacted by differences in hormonal milieu in the middle-aged cohort between sexes. Additionally, the single post-exercise training biopsy taken ~48 h after the final exercise session only captures gene and protein expression at this time point. As such, there is the potential that the last exercise session may have contributed to gene and protein expressions observed. Lastly, given this explorative study was conducted with a relatively small sample size, type I error cannot be ruled out where significant correlations were observed.

3.6 CONCLUSION

In conclusion, the results from the current study demonstrate that six weeks of single-mode exercise training increased androgen receptor protein content as well as apelin receptor and vitamin D mRNA in human skeletal muscle. Moreover, exercise training led to similar increases between modes in Akt and mTOR protein expression. A short period of detraining was insufficient to decrease androgen receptor protein and vitamin D receptor mRNA. In contrast, apelin receptor mRNA returned to baseline levels after detraining. Results from the present study suggest short-term single-mode exercise training results in similar targeted molecular responses despite different skeletal muscle training adaptations. Indeed, HIIT and ENT are capable of switching on anabolic genes and proteins suggesting other molecular transducers may be responsible for explaining more robust increases in lean mass and strength with RET. The molecular responses observed in the current study may reflect the duration of the training protocol and baseline fitness status of participants (i.e., sensitive to any contractile stimuli).

CHAPTER 4

4.1 SUMMARY

The primary aim of the investigations undertaken for this thesis was to determine the effects of short-term (six weeks) HIIT, combined with increased dietary protein availability, on skeletal muscle gene and protein expression, skeletal muscle fiber cross-sectional area (i.e., size), lean mass, muscle thickness and maximal muscle strength in sedentary, middle-aged men. The second aim was to compare these responses to RET and ENT. The final aim was to assess whether exercise training-induced adaptive responses were maintained after a short period of detraining (2.5 weeks). The major findings from the studies undertaken were:

1. Short-term exercise training increased muscle fiber size independent of modality
2. RET induced the most substantial anabolic exercise training adaptations
3. HIIT increased muscle thickness, mass and whole-body strength
4. ENT increased muscle strength despite no change in muscle thickness or mass
5. Training-induced changes in muscle size, thickness, mass and strength were largely maintained after both RET and HIIT
6. Maximal aerobic capacity remained above pre-training levels after detraining with HIIT and ENT
7. Despite divergence of physiological training adaptations, changes in the expression of measured genes and proteins were similar across exercise groups

The clinical study conducted as part of this thesis was undertaken in sedentary middle-aged men susceptible to the loss of muscle mass (i.e., pre-sarcopenia) who are an underrepresented cohort in exercise training and skeletal muscle anabolism research. The results from this thesis have clinical implications in several situations including: a) inactive overweight and obese middle-aged males seeking time-efficient exercise options that

concurrently improve muscle mass and cardiorespiratory fitness, b) pre-surgical patients with limited time to improve physical function prior to an operation, and c) clinical exercise physiologists faced with strict time constraints and/or limited resources to prescribe traditional strength-based exercise training to their clients.

A short-term exercise training duration was deliberately selected to replicate the narrow time window forced upon certain individuals prior to periods of reduced physical activity (e.g., planned surgery patients). Accordingly, a subsequent short-term period of detraining, designed to duplicate the immediate post-surgery window, proceeded exercise training in order to determine which exercise training-induced skeletal muscle adaptations were retained. Cellular, whole-muscle and whole-body adaptive responses following exercise training were examined to a) compare HIIT to ENT and RET (the ‘gold standard’ for muscle anabolism), and b) determine which exercise modality resulted in the most widespread physiological responses to support ‘all-round’ enhanced physical function (Chapter 2). The results revealed that RET and HIIT were effective for increasing all measurements indicative of muscle growth (i.e., fiber size, lean mass, muscle thickness and strength). However, only HIIT also increased peak aerobic capacity, suggesting that short-term cycling-based interval-style exercise is effective for concurrently inducing anabolic and metabolic skeletal muscle adaptations. Following detraining, improvements in aerobic capacity observed with HIIT and ENT fell below post-exercise training levels, although gains in muscle mass and strength were retained in all exercise groups. The declines in aerobic capacity are consistent with previous work investigating detraining of similar duration (~two weeks) (Coyle *et al.*, 1984). Taken together, in the face of brief exercise training cessation, short-term exercise training-induced increases in cardiorespiratory fitness are more likely to be negatively impacted than gains in muscle mass and strength.

Biochemical assays were used to determine selected exercise training- and detraining-induced changes in mRNA and protein expression of markers established, or more recently

purported, to be involved in intramuscular signalling events that form the molecular basis of skeletal muscle accrual (Chapter 3). The major findings of that investigation were that exercise training increased androgen receptor protein and mRNA expression of apelin and vitamin D receptor independent of contractile mode. This work is the first evidence that such markers are increased with HIIT and suggest they may play a role in mediating skeletal muscle anabolism in response to short-term single-mode exercise training. Previous studies have observed increased androgen receptor content in older adults after short-term (Gharahdaghi *et al.*, 2019) and prolonged RET (Sato *et al.*, 2014). The present work extends these findings to middle-aged men and short-term continuous and interval-based aerobic exercise. Furthermore, Akt and mTOR protein expression also increased following each exercise modality indicating short-term training may have enhanced ‘anabolic signalling responsiveness’ (Wilkinson *et al.*, 2008). In contrast, expression of metabolic markers related to mitochondrial and fatty acid transporter content remained unchanged suggesting that the exercise protocols (combined with increased dietary protein intake) had more potent effects on measured molecules implicated in muscle growth. To link the results described in Chapters 2 and 3, relationships between exercise-training induced changes in end point physiological measurements indicative of increased muscle mass/size (Chapter 2) and molecular markers (Chapter 3) were tested. Results from correlative analyses indicated a strong positive association between the change in leg lean mass and androgen receptor protein content in the HIIT group.

Collectively, results from the work undertaken for this thesis demonstrate that despite more widespread anabolic skeletal muscle adaptations (i.e., increased muscle fiber size, lean mass, muscle thickness and maximal strength) following short-term RET and HIIT, a uniform anabolic molecular response was observed across all exercise modalities. While short-term RET remains the ‘gold standard’ for inducing robust changes in muscle mass and strength, results from this thesis suggest that previously exercise naïve middle-aged men also experience enhanced muscle morphology and function with HIIT and, to a lesser extent with ENT.

Although HIIT-induced increases in muscle mass and strength could be considered mild compared to short-term RET (Chapter 2), future work into the anabolic effects of aerobic exercise modalities bares relevance for various populations and scenarios where time constraints are apparent but enhanced muscle mass and/or function is required.

4.2 STUDY LIMITATIONS

There are several limitations in the investigations undertaken for this thesis that require discussion when considering interpretation of the results. These include: 1) the population cohort under investigation (limited to middle-aged men therefore limiting the applicability of the findings for women (e.g., menopause-induced alterations in hormonal milieu) in the same age bracket, 2) the lack of a non-protein supplemented control group, meaning that any augmented muscle growth responses cannot be attributed to increased protein availability per se, 3) small sample size and lack of statistical power largely due to a limited number of previous studies that have assessed muscle fiber size responses following different types of short-term exercise training, 4) the lack of a true time-course of muscle biopsy sampling, therefore provides only a ‘snapshot’ of mRNA and protein expression at that point in time, 5) *vastus lateralis* ultrasound measurements were obtained in the days following a *vastus lateralis* biopsy meaning residual local swelling contributing to observed muscle thickness responses cannot be ruled out, 6) due to a lack of baseline familiarisation, a ‘learning effect’ may have contributed to observed changes in muscle strength and 7) not all participants were middle-aged given the mean age was 39 years.

4.3 CONCLUDING REMARKS

Extrapolating from the findings of this thesis, exercise physiologists working with clinical populations unable to perform RET (e.g., pain, injury, lack of access to specialised equipment) may be able to use cycling-based exercise in deconditioned populations to combat

the declines in muscle mass and strength declines that arise from reduced physical activity (e.g., following a medical procedure). Short-term HIIT and ENT should not be thought of as a replacement for RET but perhaps a substitute in scenarios where any pre-operative exercise training is better than none to attenuate potential post-surgery muscle wasting and improve post-surgery outcomes (e.g., accelerated discharge from hospital). Moreover, future longer-term studies (>12 weeks) among middle-aged adults comparing HIIT and ENT to RET are necessary to determine whether prolonged single-mode exercise training induces a skeletal muscle phenotype that promotes muscle fiber hypertrophy and gains in cardiorespiratory fitness. Indeed, any exercise modality able to induce anabolic and metabolic skeletal muscle adaptations would be attractive for promoting healthy aging through ongoing exercise training with a view to offsetting expected declines in muscle mass and cardiorespiratory fitness with advancing age. Building on the findings of this thesis, further work into older adults completing short-term HIIT as part of an exercise rehabilitation programme (pre- or post-operative) to stimulate gains in muscle mass is warranted. When considering only single-mode exercise, RET interventions comprising whole-body movements that induce sufficient stress on the cardiovascular system, while also placing muscle under high tensile loads (e.g., weights-based circuit training), may best optimise all-round physical function.

Stimulating increases in the size and proportion of type II muscle fibers in older adults through exercise and diet interventions remains a core challenge for skeletal muscle physiologists in search of combatting sarcopenia. Older adults undoubtedly feel the effects of muscle loss most harshly (i.e., reduced quality of life, increased incidence of physical inactivity-related disease) but is it a case of too little too late? While aged skeletal muscle still has the capacity to adapt in response to anabolic stimuli, the magnitude of the response is dampened, at best, compared to young adults demonstrating that a substantial period of time throughout life has been missed to attenuate inevitable reductions in muscle size, mass and function. Indeed, a long-term aspiration for the field should be to find *preventative* approaches to the

sarcopenia paradigm that can inform exercise professionals working in clinics/gymnasiums/allied health to promote healthy aging. Therefore, future work in middle-aged adults elucidating realistic lifestyle strategies that promote enhanced muscle protein accrual and physical function is warranted. While there is limited research interrogating which types of exercise (and nutrients) increase type II muscle fiber size in middle-aged populations, the findings from this thesis provide preliminary evidence that any contractile mode (combined with increased protein intake), may be beneficial for stimulating muscle fiber hypertrophy in the fight against age-related muscle loss.

APPENDIX A

A.1 SUPPLEMENTAL METHODS

A.1.1 Participant allocation to exercise training group

To randomise the allocation to exercise training, a number was assigned to each exercise training group (ENT: 1, HIIT: 2 and RET: 3) by a computer random number generator (https://www.mathgoodies.com/calculators/random_no_custom) and in blocks of three, a student researcher not involved in the project generated numbers (i.e., 1, 2 or 3) until each exercise training group had been accounted for. For example, if the same number was generated consecutively, the second number was discarded, and the process repeated until each of the three numbers appeared once. This randomisation process was repeated until an exercise training group was assigned to each envelope.

A.1.2 Maximal strength testing

Maximal upper and lower body muscle strength was assessed via a battery of one-repetition maximum (1RM) tests including bilateral leg press (45° incline), bilateral knee extension and bench press. Participants were shown correct technique for each exercise by a study researcher prior to any repetitions being performed. No weight (kg) was applied for the participant's first warm-up set to familiarise them with the verbal cues to distinguish a successful lift and safety mechanisms in place if the attempted weight was too heavy. A series of sets (3-5, 2-8 repetitions) at increasing submaximal weights were lifted until the participant reported a rating of perceived exertion (RPE) of ~16 using Borg's CR6-20 scale (Borg, 1982). For the 45° incline bilateral leg press, the 1RM attempt was deemed successful if the participant initiated the lift in ~0° knee extension, lowered the sled to ~90° knee flexion and returned the sled to ~0° knee extension. For the bilateral knee extension, the 1RM attempt was deemed successful if the participant initiated the lift in ~90° knee flexion and lifted the weight to ~0° knee extension. For the bench press, the 1RM attempt was deemed successful if the participant

initiated the lift in $\sim 0^\circ$ elbow flexion, lowered the weighted barbell until touching the chest for approximately one second and returned the weighted barbell to $\sim 0^\circ$ elbow flexion. Each 1RM attempt was followed by 5 min of rest. The 1RM for each exercise was determined in isolation (i.e., exercises were not alternated). A maximum of five 1RM attempts were allowed per exercise. The 1RM achieved for each exercise was used to determine RET intensities (%1RM).

A.1.3 Dietary intervention and analysis

Initial diet consult

At visit one of the preliminary testing period, a three-day diet recall was performed in between OGTT blood sampling. The study diet requirements were explained and participants were provided a standardised meal (45% carbohydrate, 25% protein and 30% fat; 33% of total daily energy intake (~ 3700 - 4300 kJ) based on the Cunningham equation (Cunningham, 1980)) for consumption the evening prior to a muscle biopsy. Participants were instructed to record their habitual diet for the entire preliminary testing period (~ 14 days) using a smartphone application (EasyDietDiary (iOS) or MyFitnessPal (Android)).

Follow up diet consult

At visit four of the preliminary testing period (i.e., the week prior to commencing exercise training), a follow up diet consult was conducted where participants met with the study nutritionist to receive daily energy (kJ) and protein ($\text{g}\cdot\text{kg BW}^{-1}$) intake targets for the remainder of the study.

A.1.4 Endurance exercise training protocol

Table A.1 Week-by-week overview of endurance exercise training protocol.

Week 1				
Stage 1	Session #	1	2	3
	Intensity (%MAP)	60	50	60
	Sets	6	1	6
	Reps (min)	5	8	5
	Rest (min)	1	0	1.5
Stage 2	Intensity (%MAP)	60		
	Sets	1		
	Reps (min)	5		
	Rest (min)	0		
Stage 3	Intensity (%MAP)	70		
	Sets	1		
	Reps (min)	3		
	Rest (min)	0		
Repeat each stage once				
Week 2				
Stage 1	Session #	1	2	3
	Intensity (%MAP)	60	50	60
	Sets	7	1	7
	Reps (min)	5	10	5
	Rest (min)	1	0	1.5
Stage 2	Intensity (%MAP)	60		
	Sets	1		
	Reps (min)	5		
	Rest (min)	0		
Stage 3	Intensity (%MAP)	70		
	Sets	1		
	Reps (min)	3		
	Rest (min)	0		
Repeat each stage once				
Week 3				
Stage 1	Session #	1	2	3
	Intensity (%MAP)	60	50	60
	Sets	8	1	8
	Reps (min)	5	10	5
	Rest (min)	1	0	1.5
Stage 2	Intensity (%MAP)	60		
	Sets	1		
	Reps (min)	6		
	Rest (min)	0		
Stage 3	Intensity (%MAP)	70		
	Sets	1		
	Reps (min)	3		
	Rest (min)	0		
Repeat each stage once				
Week 4				
Stage 1	Session #	1	2	3
	Intensity (%MAP)	MAP test	50	62.5
	Sets		1	6
	Reps (min)		8	5
	Rest (min)		0	1.5
Stage 2	Intensity (%MAP)	60		
	Sets	1		
	Reps (min)	5		
	Rest (min)	0		
Stage 3	Intensity (%MAP)	65		
	Sets	1		
	Reps (min)	3		
	Rest (min)	0		
Stage 4	Intensity (%MAP)	70		
	Sets	1		
	Reps (min)	3		
	Rest (min)	0		
Repeat each stage once				
Week 5				
Stage 1	Session #	1	2	3
	Intensity (%MAP)	62.5	52.5	65
	Sets	7	1	7
	Reps (min)	5	10	5
	Rest (min)	1	0	1.5
Stage 2	Intensity (%MAP)	62.5		
	Sets	1		
	Reps (min)	5		
	Rest (min)	0		
Stage 3	Intensity (%MAP)	72.5		
	Sets	1		
	Reps (min)	3		
	Rest (min)	0		
Repeat each stage once				
Week 6				
Stage 1	Session #	1	2	3
	Intensity (%MAP)	62.5	55	65
	Sets	8	1	8
	Reps (min)	5	10	5
	Rest (min)	1	0	1.5
Stage 2	Intensity (%MAP)	65		
	Sets	1		
	Reps (min)	6		
	Rest (min)	0		
Stage 3	Intensity (%MAP)	75		
	Sets	1		
	Reps (min)	3		
	Rest (min)	0		
Repeat each stage once				

MAP, maximal aerobic power

A.1.5 High-intensity interval training protocol

Table A.2 Week-by-week overview of HIIT protocol.

Week 1			
Session #	1	2	3
Intensity (%MAP)	90	95	110
Reps	10	8	12
Work (min)	1	0.67	0.5
Rest (min)	1	1	1

Week 2			
Session #	1	2	3
Intensity (%MAP)	105	115	125
Reps	10	12	15
Work (min)	1	0.67	0.5
Rest (min)	1	1	1

Week 3			
Session #	1	2	3
Intensity (%MAP)	115	120	130
Reps	10	10	12
Work (min)	1	1	0.67
Rest (min)	1	1	1

Week 4			
Session #	1	2	3
Intensity (%MAP)		115	125
Reps	MAP test	10	12
Work (min)	(no training)	1	0.5
Rest (min)		1	1

Week 5			
Session #	1	2	3
Intensity (%MAP)	120	120	130
Reps	10	10	12
Work (min)	0.67	1	0.67
Rest (min)	1	1	1

Week 6			
Session #	1	2	3
Intensity (%MAP)	120	100	120
Reps	10	8	15
Work (min)	1	1	0.5
Rest (min)	1	1	1

MAP, maximal aerobic power

A.1.6 Resistance exercise training

The RET protocol included upper and lower body weight-bearing exercise using pulley machines and free weights. At the first session, all lower body exercise movements (45° incline bilateral leg press, bilateral knee extension, dumbbell stationary lunge and dumbbell step ups) were introduced. At session two, all upper body exercise movements were introduced (bench press, seated dumbbell overhead press, incline dumbbell chest press, *latissimus dorsi* pulldown and pulley seated row). After the two familiarisation sessions, training on Mondays and Fridays comprised predominantly of lower body exercise movements while Wednesday sessions predominantly comprised of upper body exercise movements. A three-minute warm-up (50 W) on a cycle ergometer preceded each RET session. Two warm-up sets (9-12 reps) were performed for either the lower (Monday and Friday: leg press, 35 and 55% of 1RM) or upper body (Wednesday: bench press, 30 and 50% of 1RM) depending on the day of the week. Sets ranged from 3-4 and repetitions from 9-12 at 60-80% 1RM. Three minutes of rest was standardised between sets for all exercise movements. Friday sessions from week 2-6 were performed to failure for all sets whereby the weight was increased at the next set (lower body exercise movements: 5-10 kg, upper body exercise movements: 2.5-5 kg) if the participant successfully completed more than 11 repetitions at the prescribed weight. Participants performed 1) sets to failure and 2) as many reps as possible at the final set for specific movements throughout the programme to repeatedly stimulate high motor unit recruitment (i.e., strength adaptations). A three-minute cool-down at 50 W on a cycle ergometer concluded each RET session.

A.1.7 Muscle thickness

Two-dimensional (2D) B-mode ultrasound (frequency, 12 MHz; depth, 8 cm; field of view, 14 x 47 mm) (GE Healthcare Vivid-/, Wauwatosa, WI) images obtained from the longitudinal axis of the muscle belly were used to determine left and right *vastus lateralis*

muscle thickness. The halfway point (i.e., mid) between the central palpable point of the greater trochanter and the lateral condyle of the femur (i.e., 50%) was used to obtain ultrasound images. Distances between anatomical landmarks (ischial tuberosity, fibula head and the greater trochanter) were recorded at baseline and used for subsequent muscle thickness measurements. Participants lay in a supine position for five minutes prior to muscle thickness measurements being obtained.

A layer of conductive gel was applied to the linear array ultrasound probe. The probe was then placed at the scanning site parallel to the muscle fascicles and perpendicular to the skin. Pressure from the probe against the skin may affect measurement accuracy (Klimstra *et al.*, 2007). Accordingly, care was taken by the assessor (RGT) to minimise pressure on the skin due to contact with the probe while measurements were taken. Where the superficial and deep aponeuroses were not parallel, the assessor adjusted the probe orientation to ensure they were parallel. Ultrasound scans were analysed using medical imaging software (MicroDicom, Version 0.7.8, Bulgaria). The distance between the superficial and deep aponeuroses of the *vastus lateralis* was used to define muscle thickness. The superficial and deep aponeurosis angles were determined as the angle between the line marked as the aponeurosis and an intersecting horizontal reference line across the captured image (Blazevich *et al.*, 2006; Kellis *et al.*, 2009). Collection and analysis of all ultrasound scans was performed in a blinded fashion by the same assessor (RGT). The assessor is reliable with intraclass correlations (ICCs) for muscle thickness ranging from 0.97 to 0.99, typical error (TE) from 0.09 to 0.22 cm, typical error as a percentage (%TE) from 1.0 to 3.9% and a minimum detectable change (MDC) from 0.25 to 0.61 cm.

Table A.3 Week-by-week overview of resistance exercise training protocol.

Week #	Weekly session #														
	1					2					3				
	Exercise	Intensity (%1RM)	Sets	Reps	Rest (min)	Exercise	Intensity (%1RM)	Sets	Reps	Rest (min)	Exercise	Intensity (%1RM)	Sets	Reps	Rest (min)
1	Incline leg press	65	2	8	3	Bench press	2	8	3	3	Incline leg press	70	3	9	3
	DB stationary lunge	65	2	8	3	Lat pull down	2	8	3	3	Bench press	70	3	9	3
	Leg extension	65	2	8	3	Shoulder press	2	8	3	3	Leg extension	70	3	9	3
	DB step ups	65	2	8	3	Pulley seated row	2	8	3	3	Pulley seated row	65	2	9	3
2	Incline leg press	70	3	10	3	Bench press	70	3	10	3	Incline leg press	75	3*	9-12	3
	Shoulder press	70	3	10	3	Incline leg press	75	3	10	3	Bench press	70	3*	9-12	3
	DB stationary lunge	65	3	10	3	Shoulder press	72.5	3	10	3	DB stationary lunge	72.5	3*	9-12	3
	Leg extension	70	3	10	3	DB step ups	70	2	10	3	Leg extension	75	3*	9-12	3
						Lat pull down	65	2	10	3					
3	Bench press	72.5	3	10	3	Incline leg press	75	3	10	3	Bench press	72.5	3*	9-12	3
	Incline leg press	75	3	10	3	Bench press	72.5	3	10	3	Incline leg press	75	3*	9-12	3
	Incline DB chest press	72.5	3	10	3	BB stationary lunge	72.5	3	10	3	Incline DB chest press	72.5	3*	9-12	3
	Leg extension	72.5	2	10	3	Leg extension	75	3	10	3	Leg extension	75	3*	9-12	3
	Pulley seated row	67.5	2	10	3	Supinated lat pull down	67.5	3	10	3	Pulley seated row	70	2*	9-12	3
4	1RM testing (no formal RET session)					Bench press	70	4	12	3	Incline leg press	75	4*	9-12	3
						Incline leg press	70	3	12	3	Bench press	70	3*	9-12	3
						Shoulder press	70	3	12	3	DB stationary lunge	75	2*	9-12	3
						DB step ups	67.5	2	12	3	Leg extension	75	4*	9-12	3
						Supinated lat pull down	65	2	12	3					3
5	Bench press	72.5	4	10	3	Incline leg press	77.5	3^	12	3	Bench press	75	4*	9-12	3
	Incline leg press	77.5	4	10	3	Incline DB bench press	75	3^	12	3	Incline leg press	77.5	4*	9-12	3
	Shoulder press	72.5	3	10	3	Leg extension	77.5	3^	12	3	Shoulder press	75	3*	9-12	3
	BB stationary lunge	72.5	3	10	3	DB step ups	72.5	2^	12	3	Leg extension	77.5	3*	9-12	3
	Dumbbell row	65	2	10	3										
6	Incline leg press	80	4^	12	3	Bench press	75	3	10	3	Incline leg press	80	4*	9-12	3
	Bench press	75	4^	12	3	Incline leg press	80	3	10	3	Bench press	75	4*	9-12	3
	DB step up	75	2^	12	3	Incline DB bench press	75	3	10	3	BB stationary lunge	80	3*	9-12	3
	Leg extension	80	4^	12	3	Leg extension	80	3	10	3	Leg extension	80	4*	9-12	3

1RM, one-repetition maximum; reps, repetitions; BB, barbell; DB, dumbbell; lat, latissimus dorsi; *, sets performed to failure; ^ as many repetitions as possible on the last set.

APPENDIX B

Callahan, M.J., Parr, E.B., Hawley, J.A., Camera, D.M. Can high-intensity interval training promote skeletal muscle anabolism? *Sports Medicine* (2021). doi: 10.1007/s40279-020-01397-3.

The published review article cited above can be accessed at the link below.

<https://link.springer.com/article/10.1007/s40279-020-01397-3#citeas>

APPENDIX C

C.1 RESEARCH PORTFOLIO

In accordance with Australian Catholic University Higher Degree Research policies for the degree of Doctor of Philosophy with publication, the following Research Portfolio has been included to summarise and clearly identify the nature and extent of the intellectual input contributed to research outputs by the candidate and any co-authors.

C.1.1 Statement of contribution

Callahan, M.J., Parr, E.B., Hawley, J.A., Camera, D.M. Can high-intensity interval training promote skeletal muscle anabolism? *Sports Medicine* (2021). doi: 10.1007/s40279-020-01397-3.

I acknowledge that my contribution to the above paper is 70 percent.

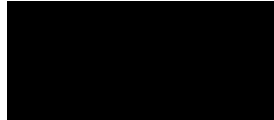
M.J. Callahan:



Date: 01/02/2021

I acknowledge that my contribution to the above paper is 10 percent.

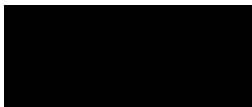
E.B. Parr:



Date: 01/02/2021

I acknowledge that my contribution to the above paper is 5 percent.

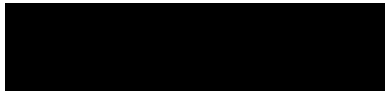
J.A. Hawley:



Date: 01/02/2021

I acknowledge that my contribution to the above paper is 15 percent.

D.M. Camera:



Date: 01/02/2021

APPENDIX D

D.1 REFERENCES

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