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**THE EFFECTS OF AEROBIC EXERCISE ON HUMAN SKELETAL MUSCLE
ADAPTATIONS TO RESISTANCE EXERCISE**

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ABSTRACT

Aerobic exercise (AE) may interfere with muscle adaptations induced by resistance exercise (RE). Three experimental campaigns were conducted to explore the influence of AE on molecular, functional and muscular adaptations to acute and chronic RE. Twenty-nine men performed unilateral knee extensor RE preceded by AE (AE+RE). The contralateral leg did RE only. First, the influence of acute AE on muscle molecular responses to RE performed 6 h later was studied. Subsequently, this exercise regimen was implemented over 5 weeks training. The relationships between acute and chronic outcomes were examined and molecular responses to acute exercise were assessed in untrained and trained muscle. Finally, acute and chronic responses to AE+RE, interspersed by only 15 min recovery, were investigated.

Phosphorylation of mTOR and p70S6K was greater after AE+RE than after RE. In parallel, myostatin was suppressed for a longer time after AE+RE. These results suggest that AE+RE enhance skeletal muscle anabolic environment more than RE alone (*Paper I*). After 5 weeks training, improvements in muscle strength and power were similar across legs. However, AE+RE prompted a greater increase in muscle size than RE, suggesting that AE potentiates the hypertrophic stimulus to RE training without altering muscle function progress (*Paper II*). Consistent with changes in whole-muscle size, AE+RE showed greater anabolic molecular responses than RE. As chronic training blunted this effect, it appears that AE offers a synergistic hypertrophic stimulus to RE only during short-term training (*Paper III*). Although putative regulators of hypertrophy such as p70S6K, myostatin and PGC-1 α 4 were examined, no molecular marker correlated with changes in muscle size, strength or power induced by training. Hence, this study challenges the concept that single molecular markers are viable predictors of training-induced muscle adaptations (*Paper III–IV*). When recovery time between exercise bouts was reduced to 15 min, AE+RE still produced a more substantial increase in muscle size than RE. However, progression of concentric strength was blunted. Thus, while restored muscle function between exercise bouts is a prerequisite for achieving maximal gains in strength and power, incomplete recovery appears not to compromise muscle hypertrophy (*Paper V*).

Collectively, the results suggest that outcomes of AE+RE are impacted by chronic training and time allowed for recovery between exercise modes. Yet, the current study offers no support to the view that AE interferes with muscle hypertrophy induced by RE.

Key words: concurrent training, endurance, gene expression, hypertrophy, muscle strength and power, protein phosphorylation

SAMMANDRAG

Uthållighetsträning kan försämra muskelns anpassning till styrketräning. Tre serier av experiment genomfördes för att undersöka effekter av uthållighetsträning på molekylär, funktionell och muskulär anpassning till akut och kronisk styrketräning. Tjugonio män utförde enbensstyrketräning som föregåtts av uthållighetsarbete (AE+RE). Det andra benet utförde enbart styrketräning (RE). Först undersöktes effekten av uthållighetsträning på molekylär respons vid styrketräning utförd 6 timmar senare. Sedan implementerades samma träningsmodell under 5 veckors träning. Förhållandet mellan akut respons och kronisk anpassning studerades, och akut molekylär respons undersöktes i otränad och tränad muskel. Slutligen studerades akuta och kroniska effekter av AE+RE, där träningspassen separerats med endast 15 minuters återhämtning.

Fosforylerat mTOR och p70S6K var högre efter AE+RE jämfört med efter RE. Samtidigt var myostatin nedreglerat under längre tid efter AE+RE. Dessa resultat tyder på att kombinerad uthållighets- och styrketräning förstärker muskelns anabola miljö mer än enbart styrketräning (*Artikel I*). Efter 5 veckors träning hade muskelstyrka och power ökat likartat i bägge ben. Trots det ledde AE+RE till större ökning av muskelsvolym än RE. Uthållighetsarbete synes därmed förstärka det stimuli för muskeltillväxt som noteras vid enbart styrketräning, utan att progression av muskelfunktion påverkas (*Artikel II*). I linje med förändringarna på helmuskelnivå noterades en mer utpräglad anabol molekylär respons efter AE+RE jämfört med efter RE. Eftersom denna effekt var avtrubbad i tränad muskel, verkar det som att uthållighetsarbete är ett synergistiskt stimuli för muskeltillväxt endast under en kortare period (*Artikel III*). Trots att påstådda reglerare av hypertrofi såsom p70S6K, myostatin and PGC-1 α studerades, korrelerade ingen molekylär markör med förändringar i muskelstorlek, styrka eller power efter träning. Därmed uttrycks tvivel om att enskilda molekylära markörer kan förutspå muskelns anpassning till träning (*Artikel III–IV*). När återhämtning mellan passen reducerades till 15 minuter, ledde AE+RE fortfarande till en större ökning av muskelvolym än RE. Dock försämrades koncentrisk styrkeutveckling av det föregående uthållighetspasset. Dessa resultat understryker vikten av att återställa muskelns prestationsförmåga mellan respektive träningspass för nå maximal utveckling av styrka och power. Ofullständig återhämtning verkar däremot inte försämra muskelhypertrofi (*Artikel V*).

Sammantaget visar dessa studier att resultatet av uthållighets- och styrketräning påverkas av kronisk träning och tid ägnad till återhämtning mellan respektive träningsform. Däremot finnes inget stöd för att uthållighetsträning begränsar muskeltillväxt vid styrketräning.

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LIST OF PAPERS

This thesis is based on the following papers, herein referred to by their Roman numerals:

- Paper I* **Lundberg TR**, Fernandez-Gonzalo R, Gustafsson T, Tesch PA. Aerobic exercise alters skeletal muscle molecular responses to resistance exercise. *Medicine and Science in Sports and Exercise* 2012; 44:1680–8.
- Paper II* **Lundberg TR**, Fernandez-Gonzalo R, Gustafsson T, Tesch PA. Aerobic exercise does not compromise muscle hypertrophy response to short-term resistance training. *Journal of Applied Physiology* 2013; 114:81–9.
- Paper III* Fernandez-Gonzalo R, **Lundberg TR**, Tesch PA. Acute molecular responses in untrained and trained muscle subjected to aerobic and resistance exercise training versus resistance training alone. *Acta Physiologica* 2013; 209:283–94.
- Paper IV* **Lundberg TR**, Fernandez-Gonzalo R, Norrbom J, Fischer H, Tesch PA, Gustafsson T. PGC-1 α expression and exercise-induced human muscle hypertrophy. *Acta Physiologica* 2014 (in review).
- Paper V* **Lundberg TR**, Fernandez-Gonzalo R, Tesch PA. Exercise-induced AMPK activation does not interfere with muscle hypertrophy in response to resistance training in men. *Journal of Applied Physiology* 2014; 116:611–20.

In addition, some unpublished data are presented.

ABBREVIATIONS

AE	Aerobic exercise
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
BF	Biceps femoris
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CS	Citrate synthase
CSA	Cross-sectional area
DNA	Deoxyribonucleic acid
eEF2	Eukaryotic elongation factor 2
FAK	Focal adhesion kinase
FOXO	Forkhead box O
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinase
MGV	Mean grey value
miRNA	Micro ribonucleic acid
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mTOR	Mechanistic target of rapamycin
MuRF-1	Muscle RING-finger protein-1
NAD	Nicotinamide adenine dinucleotide, oxidised
NADH	Nicotinamide adenine dinucleotide, reduced
NaOH	Sodium hydroxide
PA	Phosphatidic acid
PBST	Phosphate-buffered saline with tween
PFK	Phosphofructokinase
PGC-1 α	Peroxisome proliferator-activated receptor- gamma coactivator 1 alpha
PVDF	Polyvinylidene difluoride
p70S6K	p70S6 kinase
QF	Quadriceps femoris
qPCR	Quantitative polymerase chain reaction
RE	Resistance exercise
RF	Rectus femoris
RIPA	Radioimmunoprecipitation assay
RM	Repetition maximum
RNA	Ribonucleic acid
RPE	Rating of perceived exertion
rpS6	Ribosomal protein s6
RT	Room temperature
SD	Standard deviation
STARS	Striated muscle activator of Rho signalling
VEGF	Vascular endothelial growth factor
VI	Vastus intermedius
VL	Vastus lateralis
VM	Vastus medialis
W_{\max}	Maximal workload
4E-BP1	Eukaryotic initiation factor 4E binding protein 1

INTRODUCTION

Human skeletal muscle is a highly plastic tissue allowing for marked yet reversible phenotypic changes in response to altered use. Exercise training induces muscle adaptations that persist for extended periods of time. These modifications, i.e. chronic adaptations, are the accumulated result of repeated acute exercise sessions [65]. Thus, the muscle gradually adapts to repetitive exercise, and after years of specific training, displays a distinct phenotype reflecting the primary overload-stimulus imposed. Chronic endurance exercise, characterised by high-volume/low-force muscle actions, produces e.g. increased mitochondrial and capillary density [11, 30, 96]. Conversely, resistance exercise, i.e. low-volume/high-force actions, results in muscle hypertrophy and increased strength and power [158, 193]. These typical exercise adaptations represent opposite ends of the adaptation continuum [44] and are manifested in its extremes by e.g. the marathon runner displaying an exceptionally fatigue-resistant muscle profile, and the powerlifter showing extraordinary whole-muscle size.

Sports such as football, rugby, boxing, and decathlon require the athlete to simultaneously possess and train for divergent performance qualities such as strength, power, endurance and speed. Apart from athletic performance, training with the goal of enhancing both strength and endurance is vital in e.g. injury rehabilitation, health promotion, aging, or in individuals calling for effective exercise countermeasures to combat muscle deconditioning (e.g. astronauts). Muscle adaptations to these combined exercise regimens typically reside somewhere in between the extremes of the adaptation continuum. From a conceptual view, it appears unlikely that skeletal muscle is capable of concurrently achieving the extreme features displayed by the powerlifter and the marathon runner. Inevitably, the question has been raised whether skeletal muscle could comply with aerobic and resistance training simultaneously, without compromising the desired adaptations.

The effects of combined aerobic and resistance training, commonly referred to as concurrent training, was originally investigated by Robert Hickson in 1980 [94]. In that study, 10 weeks of concurrent aerobic and resistance training resulted in reduced gains in muscle strength compared with resistance training alone. This pioneer work suggested that aerobic exercise hampers muscle adaptations to resistance exercise. Since then, numerous studies have explored muscle adaptations to concurrent training. However, to fully grasp the nature of this interference effect, the rather distinct muscle adaptations that occur in response to aerobic and resistance training respectively, should be acknowledged.

Muscle adaptations to resistance exercise

The history of resistance training is often traced to ancient Greece and the legend of Milo of Croton [188]. More anecdotal evidences include the lifelong efforts by fitness advocates such as Joe Weider and Jack LaLanne, and at a more scientific level, DeLorme emphasising resistance exercise as a viable method to restore muscle power and function in patients suffering from weakness [57]. Today, it is established and common knowledge that resistance exercise increases muscle mass and strength, and hence employed by athletes to improve sport-specific performance, and by the general population to promote health, lean body mass and general fitness. Resistance could be produced by means of free weights, machines, elastic bands or one's own body weight. It is generally held coupled concentric and eccentric muscle actions ought to be executed over a number of sets and repetitions, and performed a few to several times per week depending on the goal of the trainee [1]. The acute resistance exercise stimulus is impacted by the specific mode, intensity, frequency and volume of training. Likewise, the programme design influences the subsequent adaptations to chronic training. Therefore, the myriad of possible programme variations and hence end-point results should be appreciated.

Acute responses

Muscle force production is proportional to the number of cross-bridges formed by the myofibrillar proteins actin and myosin. With increased loading, progressively more high-threshold motor units and hence fibres are recruited according to the size principle of motor unit activation [93]. Despite the high forces and power produced, resistance exercise is performed at a relatively low energy cost. At the most, and when exercise involves large muscle groups operating across multiple joints, oxygen uptake rarely exceeds 50–60% of maximal aerobic power [64]. Despite the low energy demand, a typical resistance exercise session aimed at promoting muscle accretion mobilises all major metabolic systems. Hence, muscle phosphocreatine, glycogen and triglyceride utilisation is significant, and blood and intra-muscular lactate accumulation marked [69, 194]. Resistance exercise performed at high- and moderate loads shows significant glycogen depletion of both slow- and fast-twitch fibres [198]. Acute resistance exercise also elicits an endocrine response that increases receptor interactions and modifies protein metabolism of muscle [52]. More specifically, local and systemic hormones such as testosterone, growth hormone and insulin-like growth factors are secreted and various cytokines are released [124]. It has become evident, however, that the muscle growth response is intrinsic to skeletal muscle and hence independent of circulating growth factors [187, 206, 209]. Of particular importance are the

intracellular signalling cascades that favour increased protein synthesis in response to resistance exercise (see below).

Chronic effects of resistance training on strength and power

Numerous studies show that resistance exercise promotes increased maximal voluntary strength [87, 178]. Enhanced force production is primarily mediated through increases in muscle size (i.e. hypertrophy) and neural adaptations favouring greater and more efficient muscle use [68, 137]. It is acknowledged that the neural factors, such as enhanced neural drive and inter-muscular coordination, are substantial during the early stages of training and most likely continue to contribute to strength gains in long-term training paradigms followed by e.g. olympic-style weightlifters [158, 178]. As the present work employs a resistance training regime aimed at increasing muscle size, neural mechanisms that may contribute to increased strength or power, are not discussed or reviewed in detail.

Despite a plethora of resistance training studies, there is still considerable debate regarding the most effective training programme to improve strength. While e.g. the American College of Sports Medicine advocates heavy loading (85–100% of 1 RM) and multiple sets to promote increased force [1], these recommendations have rightfully so been challenged [33, 38, 39]. Nonetheless, and consistent with the simplistic view that neural adaptations predominate early on, increases in strength are substantial during the first weeks to months of a resistance training programme [158, 178]. Later (a few to several months), muscle size and strength increases in parallel [152, 193]. Athletes exposed to long-term vigorous resistance training show extraordinary muscle hypertrophy [114, 193, 196].

Muscle hypertrophy

Resistance training induced increases in muscle mass are evident from studies employing magnetic resonance imaging (MRI), computerised tomography and ultrasound techniques [73]. In theory, any increase in muscle cross-sectional area (CSA) could be due to increases in fibre size and/or fibre number (hyperplasia). However, while the major contributor of muscle growth until early infancy is an increase in fibre number, adult muscle hypertrophy predominantly occurs through an increased protein content and CSA of individual fibres [137]. In particular, resistance training increases area and number of myofibrils of the fast-twitch type II fibres [73, 200]. Myofibrils occupy about 80% of the fibre volume in human skeletal muscle [66], with the most abundant myofibrillar proteins being myosin and actin. Thus, fibre hypertrophy occurs through *de novo* synthesis and accumulation of new myofilaments, with concomitant expansion of fibre volume

[8, 43, 136]. It is also apparent that resistance training promotes phenotype change towards an increased percentage of type IIa fibres at the expense of type IIx fibres, whereas the proportion of type I fibres remains rather unchanged [5, 189]. Morphological changes that may occur in response to resistance training include an increased fibre pennation angle [3, 73]. While this adaptation exaggerates the increase in physiological relative to anatomical CSA [3], both measures are good correlates of whole-muscle force production [17].

Protein synthesis and degradation

The ratio of protein synthesis and degradation determines muscle protein balance. When muscle protein synthesis exceeds breakdown, the net protein turnover is positive and accretion of muscle proteins occurs. During acute resistance exercise, both protein synthesis [41] and breakdown [170] are stimulated such that the net balance is negative [125]. However, when essential amino acids are consumed post-exercise, the net protein balance turns positive. Resistance exercise is capable of increasing muscle protein synthesis above baseline for 48–72 h. The elevation in muscle breakdown appears to be briefer and less robust (24 h) [170]. Thus the quantity of contractile proteins reaches a new steady-state level through the cumulative effect of repeated exercise bouts and feeding. When repeated over an extended period of time, this summates into muscle hypertrophy [13].

The protein synthetic response induced by resistance exercise is, in part, load and volume dependent [32, 34], suggesting that both factors are implicated in stimulating muscle hypertrophy. Albeit not always appreciated, high-volume aerobic exercise also increases protein synthesis [89, 186], and low-loading strategies repeated until exhaustion promote muscle growth [150]. However, while any novel exercise bout induces global protein synthesis of both myofibrillar and sarcoplasmic protein fractions, chronic training blunts this response [208], resulting in an attenuated rise in protein synthesis in the trained state [116, 191]. These findings suggest that cellular responses to aerobic and resistance exercise becomes more specific to the stimulus applied, eventually promoting contractile protein accretion more so following resistance than aerobic exercise [125].

Mechanotransduction

It is apparent that the skeletal muscle must possess a sensor(s) that transduces active or passive tension into a cellular event favouring anabolism [88]. While metabolic perturbations such as altered calcium flux and redox- and phosphorylation state may be of importance for this to occur [44], recent research highlights the membrane lipid phosphatidic acid (PA) and the protein complex focal adhesion kinase (FAK) as potential key players [53, 216]. Acute loading disturbs sarcolemma integrity and induces PA, leading to the activation of

signalling cascades that regulates protein synthesis [203]. The expression of FAK increases in response to functional overload and decreases after unloading, suggesting that FAK senses mechanical loading [70, 80, 217]. Collectively, it appears that PA and FAK are crucial in converting mechanical strain into an appropriate intracellular response initiating protein synthesis and muscle growth.

Transcriptional control

Transcriptional regulation of muscle hypertrophy has received attention as new techniques allow for the analysis of genome-wide responses to exercise [169, 201]. It is well established that resistance exercise alters the expression of genes involved in diverse functions such as cell growth and differentiation, inflammation, and proteolysis [88]. While mRNA-levels typically return to baseline within 24 h after resistance exercise [213], cross-sectional studies suggest that a few genes involved in regulating muscle mass may be chronically altered after long-term training [174, 190]. As longitudinal studies are in conflict [74, 85], the role of basal transcriptional activity in regulating muscle mass warrants further exploration.

The gene expression response to acute resistance exercise is implicated in controlling muscle protein turnover [44]. Among markers exerting transcriptional control is myostatin, a transforming growth factor- β family member functioning as a negative regulator of muscle size. Over-expression of myostatin reduces muscle size [175], and conversely, myostatin-deficient animals and humans display remarkable muscle mass [145, 184]. Acute resistance exercise diminishes myostatin expression [134] and hence aids in promoting muscle hypertrophy by reverting its inhibitory effect on muscle protein synthesis and satellite cell activity [44, 204].

The ubiquitin ligase proteins atrogin-1 and MuRF-1, activated by FOXO transcription factors, target contractile elements for degradation and hence promote muscle atrophy [26, 109]. However, while MuRF-1 and atrogin-1 are linked to muscle breakdown in response to disuse models, the role of these markers in healthy skeletal muscle remains poorly understood. MuRF-1 increases in response to resistance exercise [134, 139] whereas atrogin-1 remains unchanged [214] or decreases [139]. Interestingly, recent reports suggest that MuRF-1 facilitates tissue remodelling in favour of muscle growth [54, 104].

PGC-1 α is a transcriptional co-activator implicated in mitochondrial biogenesis [106]. However, the physiological function of this gene may be isoform-specific [177]. Indeed, it has been proposed that a novel splice variant, termed PGC-1 α 4, controls muscle hypertrophy in response to resistance training [177]. This isoform is produced by alternative 3' splicing, whereby a stop codon is introduced, resulting in a protein shorter (truncated) than the full-length PGC-1 α . While these

findings remain to be further scrutinised, PGC-1 α 4 has emerged as a tentative key regulator of muscle hypertrophy *in vivo*.

The possible role of microRNAs (miRNA) in the muscle adaptive response to exercise has been acknowledged [56, 95]. miRNAs are non-coding sequences of mRNA that degrades target transcripts by binding to complementary sequences [88]. It has been estimated that 30% of the genome involves miRNA processing, and over-expression has the potential to markedly influence the transcription profile [95]. Several miRNAs increase in response to resistance exercise and may take part in regulating training-induced phenotypic changes [56, 117]. Their precise roles in these processes are, however, uncertain.

Myonuclei addition

During hypertrophy, myonuclei addition could aid in maintaining the myonuclei-to-cytoplasmic ratio intact and hence protect the capacity for transcription [7, 160]. The addition of new nuclei to existing fibres is mediated through the proliferation of satellite cells [207]. The importance of satellite cell activity and myonuclei addition in the hypertrophic process is under debate [111, 112, 127, 141, 202]. Myonuclei content was greater in powerlifters than in untrained controls [110], and fibre hypertrophy induced by resistance training was paralleled by increased myonuclei content [112] and satellite cell number [168]. Conversely, hypertrophy was accompanied by gradually decreased myonuclei domain during 90 days resistance training [111], and in mouse skeletal muscle, robust muscle growth is possible even in the absence of satellite cells [141]. Taken together, it appears that at least modest changes in muscle size are possible without the addition of new myonuclei [111].

Intracellular signalling responses

Several signalling pathways appear to be involved in the control of muscle mass; i.e. calcineurin and cytokine signalling, mitogen-activated protein kinases (MAPK), striated muscle activator of Rho signalling (STARS) and the insulin-like growth factor route [44, 76, 126]. Exercise-induced increases in protein synthesis are predominantly due to augmented translation rather than increased mRNA quantity [211]. Translating mRNA into protein includes the processes of initiation, elongation and termination [29]. Of particular importance for this process is the initiation step, which is coordinated by the mechanistic target of rapamycin (mTOR) complex [151]. mTOR integrates signals from mechanical stimuli, energy status and nutrients, to coordinate downstream signalling events [101]. Indeed, activation of mTOR elicits increased protein synthesis after acute resistance exercise [60], and is crucial for the muscle hypertrophic response [25, 27]. Thus, inhibited mTOR activity blunts protein synthesis [61] and muscle hypertrophy

[27]. Downstream effector targets of mTOR include p70S6 kinase (p70S6K), eukaryotic initiation factor 4E binding protein 1 (4E-BP1), ribosomal protein S6 (rpS6) and eukaryotic elongation factor 2 (eEF2). Numerous studies support their role in augmenting protein synthesis in response to resistance training [140, 205]. For example, p70S6K was associated with gains in muscle mass following high-frequency electrical stimulation in rats [15], and after 14 weeks resistance training in humans [192]. Moreover, levels of mTOR signalling were paralleled by increases in protein synthesis after acute resistance exercise and muscle accretion after chronic training [140]. Thus, translational control of protein synthesis is generally held as the most crucial molecular event regulating muscle size [205].

Muscle adaptations to aerobic exercise

Endurance sports such as long-distance running, road cycling and cross-country skiing rely primarily on aerobic metabolism and training aims at sustaining a high power output over an extended period of time. This is achieved through multiple central and local physiological adaptations. Increased maximal oxygen uptake is accomplished mainly through increased cardiac output [67], and manifests in high-calibre endurance athletes displaying remarkably high aerobic power [180]. Work capacity is further augmented through peripheral muscle adaptations such as increased muscle oxidative enzyme activity, mitochondrial and capillary density, and intracellular lipid stores [11, 90, 96, 97, 100].

Acute responses

The increased energy requirement typical of aerobic exercise triggers circulatory, respiratory and hormonal responses. At the muscle level, increased blood flow and metabolic perturbations, such as a drop in pH, increase in oxygen demand and accumulation of AMP, calcium and free radicals [65, 71], are evident. These primary stressors activate secondary messengers and signalling molecules such as MAPK and AMP-activated protein kinase (AMPK) to initiate gene transcription and downstream signalling events [44, 90]. Thus, unlike the adaptive responses to resistance exercise, peripheral adaptations to endurance exercise are primarily controlled at the transcriptional level [65]. Similar to resistance training, however, the end-point training outcome depends on the specific exercise mode, intensity, frequency and volume.

Mitochondrial biogenesis

Mitochondria increase in size and number in response to endurance training [79]. Mitochondrial content may increase in both subsarcolemmal and intermyofibrillar regions [99] and in all three fibre types [102]. In parallel, training augments mitochondrial oxygen uptake and enzyme activity [97]. Hence, the increase in mitochondrial volume is linearly related to increased oxidative capacity and endurance performance [100], emphasising the importance of mitochondrial biogenesis in training-induced improvements of metabolic function and muscle oxidative capacity.

PGC-1 α is a key regulator of mitochondrial biogenesis. PGC-1 α activates various transcription factors, which results in a coordinated expression of mitochondrial proteins encoded from nuclear and mitochondrial DNA [132]. Single exercise bouts up-regulate PGC-1 α mRNA and protein content [16, 106, 171], and over-expression of PGC-1 α promotes mitochondrial biogenesis and increased oxygen uptake and

exercise capacity [212]. Thus, PGC-1 α is a master regulator of mitochondrial biogenesis in response to aerobic training [172].

Angiogenesis

Endurance training induces skeletal muscle capillary proliferation [11]. The expansion of the capillary network often occurs in parallel with increased maximal oxygen uptake and is important to meet the higher rate of blood flow and hence oxygen delivery to the working muscle [181]. Increased capillarity is accomplished through both sprouting and non-sprouting mechanisms, and stimulated by factors such as increased blood flow, shear-stress and metabolic perturbations [82]. Regardless of the triggering factor, the vascular endothelial growth factor (VEGF) is a predominant regulator of angiogenesis [83, 164]. VEGF increases in response to acute exercise and acts to stimulate angiogenic processes [84]. In further supporting the role of VEGF, muscle deficiency in VEGF attenuates training-induced angiogenesis and performance improvements [163, 164].

Concurrent training

Although the skeletal muscle adaptations outlined above represent classical end-point adaptations to resistance and aerobic training, it should be acknowledged that phenotype changes occur along a continuum and several muscle adaptations show minor specificity across exercise modes. For example, muscle fibre size may increase in response to aerobic training [11, 78] and myosin heavy-chain transformation occurs in the same direction regardless of exercise modality [123]. Moreover, high-volume resistance training may induce capillary proliferation [183] and increased oxidative enzyme activity [197]. These examples highlight the complexity of the muscle adaptive response to exercise. At the cell level such effects are likely influenced by cross talk, redundancy and feedback loops. Thus, given the limited understanding of the mechanisms dictating classical adaptations to aerobic and resistance exercise, it is perhaps not surprising that the mechanisms regulating adaptation to concurrent exercise are poorly explored.

The effects of aerobic exercise on muscle adaptations to resistance exercise

The integration of aerobic and resistance exercise in a training programme has been termed concurrent training. Since the work of Hickson [94], numerous studies have investigated various performance indices after concurrent training compared with specific exercise modes. Collectively, the evidence suggests that resistance training does not alter maximal oxygen uptake, yet boosts endurance performance when performed concurrently with aerobic training [2, 18, 131]. Results from studies examining the effects of aerobic exercise on adaptations to resistance exercise are equivocal. It is often held, however, that adding aerobic exercise to the resistance training programme compromises important strength outcomes [14, 91, 155]. Thus, muscle hypertrophy, maximal strength and power, sprint and jump performance, and explosive strength and rate of force development, are attenuated after concurrent training [20, 42, 50, 58, 63, 77, 86, 94, 103, 123, 173]. Other studies have reported no interference [75, 130, 142, 143, 159, 185]. These discordant findings have been attributed to variations in training protocols, and exercise modalities employed [130].

Training programme variables

A prerequisite for the interference effect is that both exercise modalities engage the same muscle group [123]. Once this criterion is met, the outcome of concurrent training depends on the specific subject sample, training programme and outcome measure selected. Indeed, concurrent training studies have employed a broad range of exercise durations, intensities and frequencies of training for the respective aerobic and resistance exercise mode, making comparisons across studies difficult, if not impossible.

The training volume, dictated by duration and frequency, could readily explain some of the controversies in the concurrent training literature. Training frequencies of 2–3 days/week appear not to compromise outcomes of resistance training [130, 142, 143]. Training conducted 5–6 days/week is more likely to do so [20, 77, 94]. This seems to hold true even when the weekly training volume is matched, suggesting that the scheduling of training sessions is an independent factor to consider, irrespective of total training volume.

If aerobic and resistance training are scheduled on the same day, some [19, 35], but not all [49, 81], reports suggest that interference is minute if resistance exercise precedes aerobic exercise. Exhaustive aerobic exercise impairs neuromuscular function and reduces performance [21, 129]. Thus, to avoid residual fatigue from a previous exercise bout, aerobic and resistance exercise should be scheduled on alternate days. While this strategy allows for normalisation of muscle function between bouts, it increases the frequency (days/week) of training. As mentioned, this too may be counterproductive for optimal adaptations to occur. Therefore, scheduling contrasting exercise modes on the same day interspersed by recovery, to restore muscle function between bouts, presents an alternative attractive approach. To date, however, few studies have employed this design and hence its efficacy remains to be determined.

A recent meta-analysis quantified the importance of various training variables on the interference effect. As determined by a total of 422 effect sizes, the most severe interference was noted for peak power performance [210]. Interference was further exacerbated when the running mode of exercise was employed, and high (>3days/week) training frequencies and >20–30 min endurance training duration also negatively impacted adaptations to resistance training [210]. Although these results are relevant for individuals aiming at developing both strength and endurance, there have been few attempts to address the potential mechanisms underpinning the incompatibility of aerobic and resistance exercise.

Proposed mechanisms causing interference

Overtraining

Several early concurrent training studies employed high training volumes. Hence overtraining may simply have explained the interference effect [63]. While the muscle might struggle to comply with 10 training sessions per week (see the Hickson study), only a single study reported a more catabolic environment after concurrent training compared with resistance training alone [123]. Further, if overtraining were to compromise adaptations to concurrent training, it would selectively impair adaptations typical of resistance training, since aerobic adaptations are either augmented or unaffected by concurrent training compared with aerobic training alone [131].

Residual fatigue and low glycogen content

Aerobic exercise results in residual fatigue, which reduces the capacity for high force production during a subsequent resistance exercise bout [21, 50]. If employed chronically, muscle adaptations may be attenuated due to the reduction in day-by-day training quality [179]. Associated with the fatiguing effects of aerobic exercise is a decrease in muscle glycogen content [108]. Commencing resistance exercise with low glycogen concentrations may [51] or may not [37] alter the cellular response controlling protein synthesis. Allowing time for muscle function to recover between bouts could therefore be imperative in order to optimise adaptations to concurrent training.

Neurological factors

Impaired explosive strength, power and rate of force development have been reported after concurrent training. These outcome variables appear more susceptible to interference than pure torque/force measures [210], suggesting that neurological factors may underpin the interference effect. However, in supporting the neural hypothesis are only a few studies reporting reduced rate of force development after concurrent training accompanied by a reduction in rapid neural activation [86, 182].

Blunted hypertrophy through conflicting signalling responses

There is evidence to suggest that aerobic exercise compromises outcomes of resistance training by blunting the hypertrophic response [50, 123, 173]. While the mechanisms underlying this effect are not known, it is reasonable to assume such an interference effect would reside within the muscle. In fact recent human data suggested that the immediacy of diverse contractile activity after aerobic and resistance exercise reduces the desired intracellular signalling response produced by either mode of exercise in isolation [46]. This hypothesis accords with whole-

muscle data showing diminished hypertrophy of type I fibres (which are recruited during both aerobic and resistance exercise) after concurrent aerobic and resistance exercise, compared with after resistance training only [123, 173].

The thesis of incompatible cellular pathways originates from work with rat skeletal muscle showing divergent muscle signalling after contrasting modes of exercise [12, 156]. More specifically, muscles subjected to either high- or low frequency electrical stimulation, purported to mimic resistance and aerobic exercise respectively, showed increased mTOR phosphorylation after resistance, but not aerobic, exercise [12]. Conversely, aerobic exercise induced AMPK activation and increased protein levels of PGC-1 α . While these results infer that mode-specific signalling through AMPK and mTOR pathways dictates classical end-point adaptations to chronic aerobic and resistance training, results of human studies are equivocal. For example, when strength- and endurance-trained athletes performed their habitual or non-habitual exercise mode, robust anabolic muscle signalling was noted after the unfamiliar exercise mode only [48]. Moreover, AMPK has been reported to increase in response to resistance exercise [60], and mTOR to be induced by aerobic exercise [138]. These findings demonstrate that signalling divergence in human muscle may not be as clear-cut and distinct as in the rat.

If the understanding of exercise mode-specific signalling responses is poor, the knowledge about responses to mixed-mode or concurrent training is even more limited. In the rat, contraction-induced mTOR signalling is inhibited by prior AMPK activation through endurance exercise [199]. More specifically, translational signalling is compromised via attenuation of key downstream regulators such as 4E-BP1 and p70S6K. This is consistent with the marked suppression of protein synthesis following AMPK activation [28], and infers that recovery between bouts is critical in order to avoid the negative effects of AMPK on muscle growth. Collectively, the scenario of conflicting molecular pathways offers reasonable mechanistic support to implicate that concurrent training attenuates myofibre protein accretion. Nevertheless, it remains that currently no human study investigating the effects of concurrent training has linked adaptations noted at the whole-muscle level to mechanistic events inside the cell.

Aim and hypothesis

The overarching aim of this thesis was to study the effects of aerobic exercise on muscle responses and adaptations prompted by acute and chronic resistance exercise. The specific aims were to:

- | | |
|------------------|---|
| <i>Paper I</i> | Examine acute muscle molecular responses to resistance exercise preceded (6 h) or not by aerobic exercise |
| <i>Paper II</i> | Study muscle hypertrophy and <i>in vivo</i> muscle function in response to 5 weeks resistance training preceded (6 h) or not by aerobic exercise |
| <i>Paper III</i> | Compare acute molecular responses to resistance exercise, preceded (6 h) or not by aerobic exercise, in untrained and trained muscle and in relation to chronic adaptations |
| <i>Paper IV</i> | Investigate the expression of PGC-1 α splice variants in human muscle after acute and chronic resistance and concurrent aerobic and resistance exercise, and in relation to muscle hypertrophy |
| <i>Paper V</i> | Study acute molecular and functional responses, as well as chronic training adaptations, in response to acute and 5 weeks resistance training preceded (15 min) or not by aerobic exercise |

The overall hypothesis was that aerobic exercise would interfere with the progression of *in vivo* muscle function and hypertrophy resulting from resistance training alone.

METHODS

General design

Three experimental studies were performed (*Study I–III*; **Table 1, Fig. 1**). In each study, the effects of aerobic exercise (AE) on muscle responses and adaptations to resistance exercise (RE) were investigated. Each study employed a unilateral exercise model in which one leg was subjected to AE and RE (AE+RE), whereas the other leg served as a control and performed RE only.

In *Study I*, nine men performed one-legged AE in the morning and four sets of RE for each leg 6 h (360 min) later (AE-360-RE). Muscle biopsies were obtained from each leg before and 15 min and 3 h after the RE bout. Tissue samples were subsequently analysed to determine gene expression and translational signalling responses (*Paper I*).

Study II implemented the daily exercise regimen from the first study over 5 weeks training. Maximal strength and power were determined before and after training, and peak power was measured during all sessions. M. quadriceps femoris (QF) volume, CSA, and signal intensity were assessed by MRI. Analysis of muscle biopsies obtained at rest before and after training, determined fibre-type specific CSA, gene expression and enzyme and glycogen levels (*Paper II*). In addition, before and after training, muscle biopsies were obtained pre- and 3 h post-acute RE, with and without preceding AE (**Fig. 1**). Thus, molecular responses in untrained and trained muscle were assessed and compared across AE+RE and RE, and the relationships between acute and chronic outcomes were examined (*Paper III*). Finally, isoform-specific expression of PGC-1 α was studied in response to acute exercise and chronic training (*Paper IV*).

In *Study III*, time for recovery was reduced to 15 min, instead of 6 h, between AE and RE bouts (AE-15-RE). An acute exercise session was performed employing either AE-15-RE or RE only. Peak power was measured, and muscle biopsies obtained immediately before and 3 h after RE determined glycogen levels, gene expression and protein phosphorylation. The identical AE-15-RE vs. RE regimens were subsequently implemented in a 5-week training programme. Maximal strength and power were measured before and after training. QF volume, CSA and signal intensity were assessed by MRI, and analysis of muscle biopsies obtained at rest and 72 h after training, determined citrate synthase (CS) activity and glycogen content (*Paper V*).

Table 1. Overview of the three experimental studies carried out in this thesis

	<i>Study I</i>	<i>Study II</i>			<i>Study III</i>
Design	Acute unilateral AE+RE vs. RE.	Chronic (5 weeks) unilateral AE+RE vs. RE. Acute bouts performed before and after the training period.			Chronic (5 weeks) unilateral AE+RE vs. RE. Acute bouts performed before the training period.
Recovery between AE and RE	360 min	360 min			15 min
Subjects	N=9 men Age/height/weight: 23 (range 20–27) years 181 ± 6 cm 75 ± 6 kg	N=10 men Age/height/weight: 25 (range 20–33) years 184 ± 6 cm 83 ± 13 kg			N=10 men Age/height/weight: 26 (range 20–35) years 183 ± 7 cm 77 ± 9 kg
Publication	<i>Paper I</i>	<i>Paper II</i>	<i>Paper III</i>	<i>Paper IV</i>	<i>Paper V</i>
Key outcome measures	<ul style="list-style-type: none"> ▪ Power ▪ Gene expression ▪ Protein phosphorylation 	<ul style="list-style-type: none"> ▪ Power ▪ Torque ▪ Muscle size ▪ Immunohistochemistry ▪ Enzyme activity ▪ Gene expression 	<ul style="list-style-type: none"> ▪ Gene expression ▪ Protein phosphorylation 	<ul style="list-style-type: none"> ▪ PGC-1α isoform expression 	<ul style="list-style-type: none"> ▪ Power ▪ Torque ▪ Muscle size ▪ Enzyme activity ▪ Gene expression ▪ Protein phosphorylation

Note: AE = aerobic exercise; RE = resistance exercise

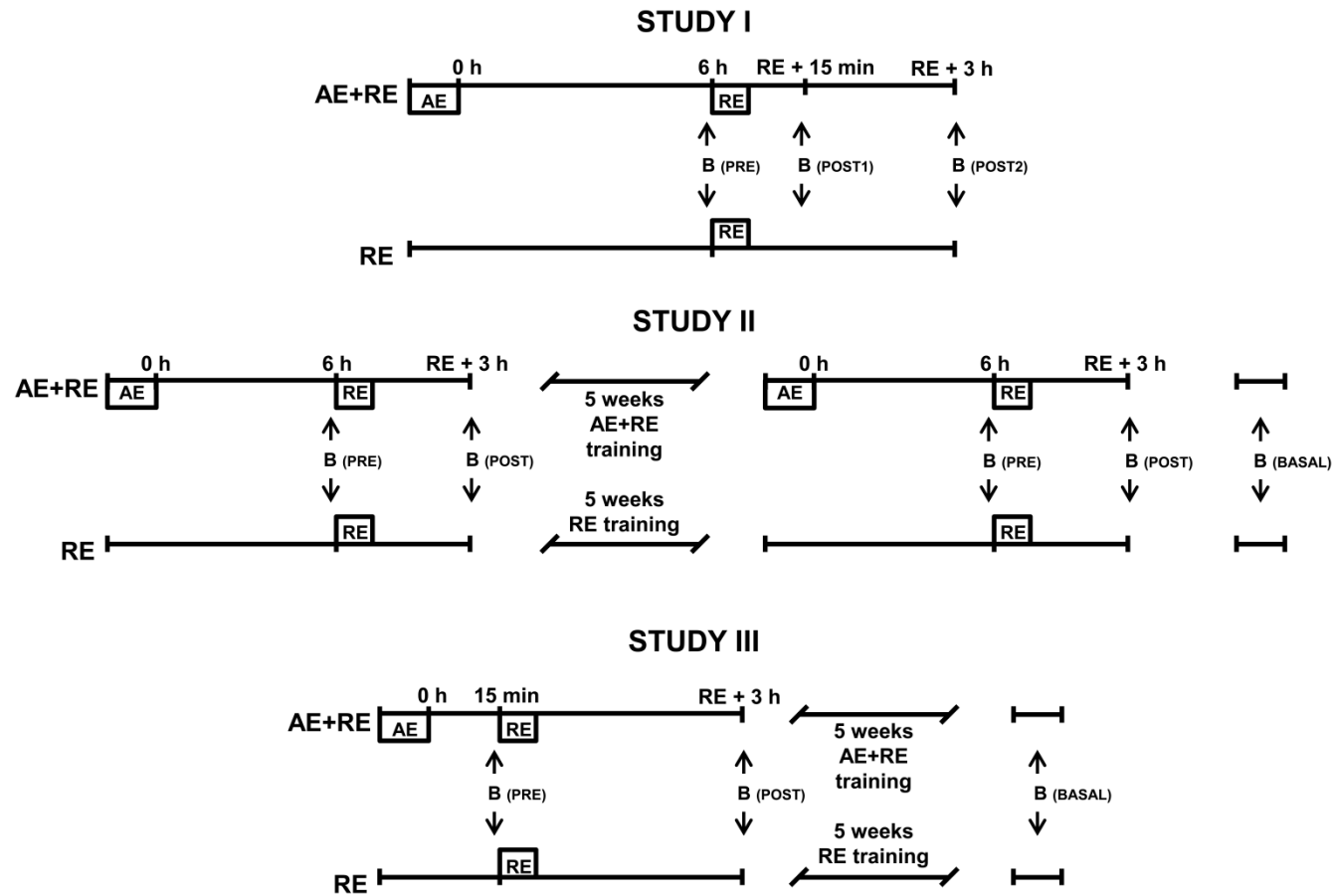


Figure 1. Experimental overview of the studies carried out in this thesis. In each study, one leg performed aerobic + resistance exercise (AE+RE), while the other leg performed resistance exercise only (RE). B = muscle biopsies.

Subjects

Subjects (**Table 1**) were university students who trained to a moderate level and who engaged in recreational activities, such as skiing and team sports, on a regular basis (2–3 days/week). They had slight to moderate experience of weight training before the study, yet had not performed regular or structured RE training during the past year. The study experiments and procedures including risks and discomforts were explained before subjects gave their informed written consent to participate. The study protocols were approved by the Regional Ethical Review Board in Umeå (*Study I* and *II*) and Stockholm (*Study III*).

Exercise equipment

One-legged aerobic exercise

Unilateral AE was carried out on a modified cycle ergometer (**Fig. 2**; model 828E, Monark Exercise AB, Varberg, Sweden) [10, 40]. This particular exercise device allows for isolated QF muscle use in the concentric mode of knee extension [10]. While seated, an adjustable bar linking the ergometer crank and a custom-made lightweight plastic boot, worn by the subjects, was set individually to allow for extensions ranging from 90° to 175° of knee angle. The flexion phase of the action was passive such that the inherent flywheel momentum repositioned the leg. Power and cadence (rpm) were sampled at 2 Hz using a wireless SRM Training System (SRM GmbH, Jülich, Germany), and analysed using associated software.

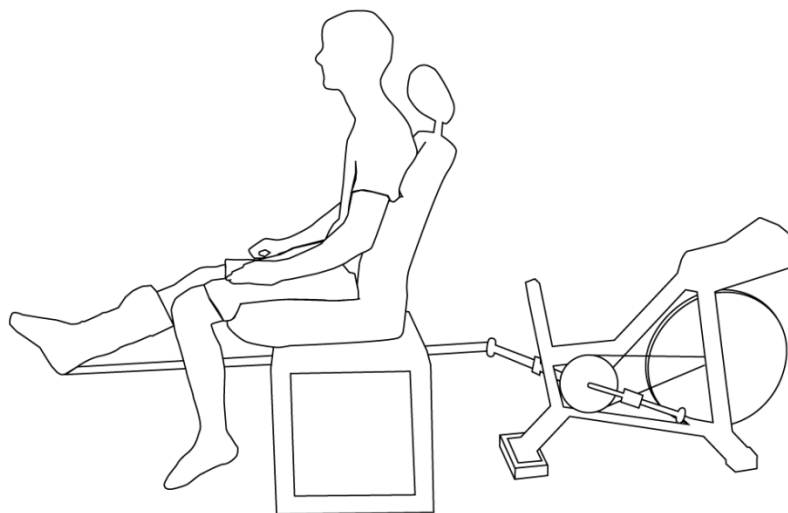


Figure 2. The one-legged cycle ergometer used as the aerobic exercise mode.

Flywheel resistance exercise

Resistance exercise emphasising QF muscle use was performed in the seated leg press (*Study I*) and knee extension mode (**Fig. 3**; *Study I-III*). Non-gravity dependent devices (YoYo Technology Inc., Stockholm, Sweden) described elsewhere [23, 195] were employed to conduct these exercises. These apparatuses use the inertia of a spinning flywheel (~4.2 kg; moment inertia 0.11 kg/m²) to offer unlimited resistance during coupled concentric and eccentric actions. Subjects were seated and slightly reclined (hip angle ~90°). The range of motion was from ~90° knee angle to near ~180° (full extension). By knowing the inertia employed, peak concentric and eccentric torque and power of each repetition were calculated by measuring rotational velocity (SmartCoach Europe AB, Stockholm, Sweden), and force (MuscleLab, Langesund, Norway).

Isokinetic torque assessment

Knee extensor maximal isometric and isokinetic torque were assessed before and after training (see later) using a Cybex II (Lumex Inc., New York, USA) dynamometer, calibrated before each test. Thigh, hip and chest were stabilised to the dynamometer chair using straps. Torque was sampled at 100 Hz using MuscleLab. For all apparatuses used, individual settings were maintained throughout the studies.

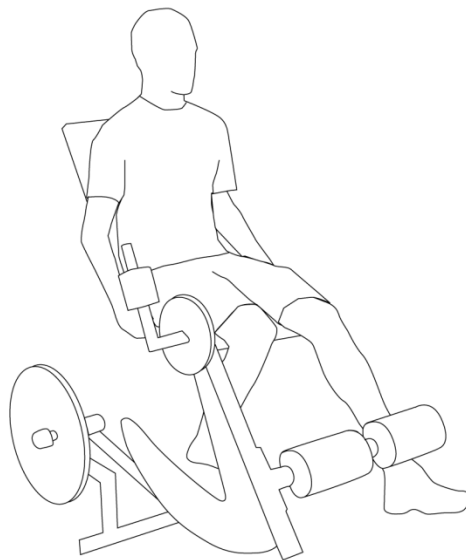


Figure 3. Flywheel knee extension ergometer used for the resistance exercise sessions.

Testing procedures

Identical test protocols were employed within a week before and after the training periods (*Study II and III*). First, MRI scans were performed (described below). During the second visit, muscle biopsies (details below) were obtained and during the third and fourth visit, muscle function was assessed. Peak isokinetic torque was measured at preset angular velocities of 30, 60, 120, 180, 210 and 270 °/s. Subjects performed two maximal actions (30 s rest) at each velocity and the best result represented peak torque. Maximal isometric torque was measured at a fixed knee angle of 120°. Further, peak torque and power were assessed on the flywheel ergometer. Subjects performed 2 x 7 repetitions with 2 min rest between sets. Strong verbal encouragement was used to call for maximal effort. Peak values were averaged across sets and repetitions. Normalised torque was calculated by dividing peak knee extension torque with average muscle CSA. An incremental test assessed maximal workload (W_{\max}) and endurance performance (time to exhaustion) on the one-legged cycle ergometer. Resistance was increased by 2.5 N every 2nd min until failure to maintain the prescribed cadence of 60 rpm. W_{\max} was defined as the last successfully completed workload. Capillary blood (20 µl) was sampled (*Study II*) from the ear lobe at rest, every 2nd min into exercise, and 1 and 3 min post-exercise. Samples were subsequently placed in a 1 ml haemolysing solution and analysed for lactate concentration (EKF-diagnostic GmbH, Magdeburg, Germany). Heart rate was recorded (Polar Electro OY, Kempele, Finland) continuously throughout the test and subsequently analysed (Polar ProTrainer 5). Rating of perceived exertion (RPE; central and local) was obtained every 2nd min, and at exhaustion using the 6–20 Borg scale [31]. Subjects were blind to any test result to ensure non-biased efforts.

Training protocols

During the 5-week training interventions, subjects completed 15 unilateral AE sessions (3 non-consecutive days/week), and 12 unilateral RE sessions for both limbs (2 days/week during week 1, 3 and 5 and 3 days/week during week 2 and 4). Hence, one limb performed concurrent AE+RE while the other limb was subjected to RE only. Legs chosen for the AE+RE intervention were randomised in a counterbalanced manner.

AE comprised 40 min continuous one-legged cycle ergometer exercise. The initial target load was 70% of the W_{\max} at a fixed cadence of 60 rpm. However, RPE (central and local) was obtained every 10th min to customise the workload such that a very strenuous effort was achieved during each exercise bout. After 40 min, the workload was increased by ~20 W and subjects were requested to continue until failure, which occurred within 1–5 min (average 2 min 30 s). Subjects received

real-time visual feedback of power and cadence via a computer monitor. Heart rate was recorded continuously during one randomly selected session each week. In the same session, capillary blood, for subsequent analysis of lactate concentration, was sampled (*Study II*) at rest, every 10th min, and, 1 and 3 min after exercise.

RE was performed 6 h (*Study II*) or 15 min (*Study III*) after completion of AE on the same day. During each session, 4 sets of 7 concentric-eccentric knee extensions were executed for each leg (alternating leg between sets; 2 min rest) in the flywheel ergometer. Subjects were requested to perform each repetition with maximal effort, and were verbally encouraged throughout each set. Peak power for each repetition was measured and subjects were supervised during all training sessions.

Acute exercise experiments

In each of the three studies, acute exercise bouts of AE+RE and RE were performed. In the morning of the experimental day, subjects performed the AE bout, i.e. one-legged cycle ergometer exercise, identically as described for training. Capillary blood was sampled for analysis of lactate concentration (*Study I and II*), and heart rate and RPE were monitored as described above. Subjects then performed RE 6 h (*Study I and II*) or 15 min (*Study III*) after completion of AE (**Fig. 1**). They executed 4 sets x 7 repetitions in the flywheel configurations. A 2-min rest was allowed between sets.

Muscle biopsies

Using the percutaneous needle biopsy technique [24], muscle samples (100–200 mg) were obtained from the vastus lateralis (VL) of each leg immediately before, and 3 h after the RE session (**Fig. 1**). In *Study I*, a biopsy from each leg was also obtained 15 min after acute RE. Furthermore, biopsies were obtained 72 h after the 5-week training periods. Following local anaesthesia administered to the skin and muscle fascia, biopsies were taken from the mid portion of the muscle through separate incisions 20 mm apart (moving distal to proximal), using a 5 mm Bergström-needle with suction applied. Samples were visually inspected, and excess blood, fat and connective tissue were removed, before frozen in liquid nitrogen and stored at -80°C until analysis.

Diet and exercise control

Subjects refrained from strenuous physical activity and alcohol for a minimum of 48 h prior to any test day. A standardised meal (pasta, tomato sauce and juice) was provided at ~8.00 pm on the night before biopsies were obtained. On the

experimental day, subjects had a standardised breakfast 2 h prior to the first biopsy procedure. Lunch consumed ~3 h prior to RE was provided between the bouts in *Study I and II*. Breakfast and lunch consisted of commercial energy drinks (Ensure Plus, Abbott Laboratories Ltd, Maidenhead, UK). Water was allowed *ad libitum* at any time. Subjects were requested to record food intake on test days, and replicate the same diet regimen on corresponding test days after the training period. The individual testing schedule was very similar (± 2 h) before and after training. Throughout the studies, subjects were instructed to maintain ordinary daily activities and routines, and to refrain from strenuous activities involving the lower limbs.

Magnetic resonance imaging

Identical MRI techniques were employed in *Study II and III*. Scans were performed before and 48–72 h after the training periods. To avoid the influence of fluid shifts on muscle size, subjects rested in the supine position for 1 h prior to scans [22]. T2 weighted cross-sectional images (50 images, 10 mm slice thickness) were subsequently obtained using a 1.5 Tesla Philips MR Systems Intera unit (Best, The Netherlands). Anatomical landmarks and standardised positioning ensured that the same segment was scanned before and after training. CSA (cm²) and signal intensity (mean grey value; MGV) of each individual QF muscle (**Fig. 4**; VL, vastus intermedius (VI), vastus medialis (VM) and rectus femoris (RF)) were analysed from the image where gluteus maximus was no longer visible and ending with the last image in which RF appeared. The signal intensity of MRI's is proportional to hydrogen concentration and is therefore used as a crude indicator of muscle water content [166]. Every third image [6] was analysed to quantify CSA and signal intensity using ImageJ software (National Institutes of Health, Maryland, USA). As an additional control, signal intensity of biceps femoris (BF) was analysed in the third image of each subject. Average CSA was multiplied by slice thickness to obtain final muscle volume.

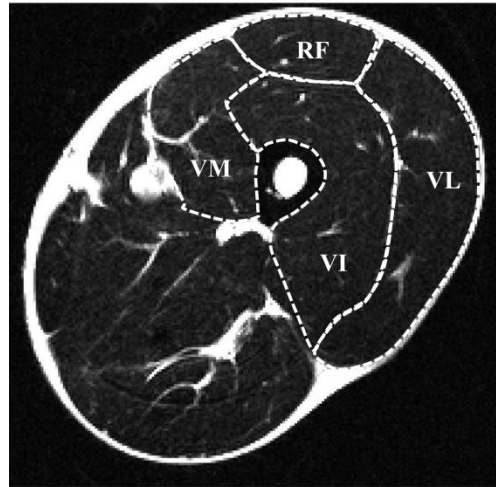


Figure 4. Cross-sectional MRI image displaying the four individual quadriceps muscles. RF = rectus femoris; VL = vastus lateralis; VI = vastus intermedius; VM = vastus medialis.

Laboratory analysis

RNA extraction, reverse transcription and real-time qPCR

In each study, about 20 mg of muscle tissue was homogenised in TRIzol, and total RNA was extracted. One μg of total RNA from each sample was used for reverse transcription into cDNA for a final volume of 20 μl (High-Capacity Reverse cDNA Transcription Kit, Applied Biosystems, Foster City, CA, USA). Gene expression (mRNA) was determined using real-time qPCR procedures (ABI-PRISMA 7700 Sequence Detector, Perkin-Elmer Applied Biosystems). Gene-specific primers and probes (TaqMan) for atrogin-1, MuRF-1, myostatin, PGC-1 α and VEGF were purchased from Applied Biosystems. GAPDH and 18S were used as reference genes. Reaction and amplification mixes (10 μl) consisted of the diluted (1:100) cDNA (4.5 μl), TaqMan Fast Universal PCR Master Mix (5.0 μl) and specific primers (0.5 μl). Subsequent cycling protocols were 2 min at 50°C and 10 min at 90°C followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Target gene expression was reported as a ratio to the reference gene using the $2^{-\Delta\text{CT}}$ formula [133].

To examine isoform-specific expression of PGC-1 α (*Paper IV*), primers designed by Ydfors et al. [215] were used to identify PGC-1 α splice variants transcribed from the proximal promoter (exon 1a; here called PGC-1 α -ex1a), and from the upstream-located alternative promoter (exon 1b; PGC-1 α -ex1b). Primers for the truncated forms of PGC-1 α (trunc-PGC-1 α) covered the insert of exon 7a and primers for the non-truncated form of PGC-1 α (non-trunc-PGC-1 α) covered the exon 6 and 7b boundaries, excluding the insert sequence. Primers covering exon 2

and 3 boundaries represented the full PGC-1 α gene (PGC-1 α total). The reaction volume included 5 μ l of 1:100 cDNA sample, 7.5 μ l SYBR Green PCR Master Mix (Applied Biosystems), 0.5 μ l of forward primers (0.4 μ M), 0.5 μ l of reverse primers (0.4 μ M) and 1.5 μ l of purified water. GAPDH was used as reference gene.

Protein extraction and western blotting

About 30 mg frozen muscle was homogenised in RIPA buffer. Twenty μ l of buffer were used per 1 mg of tissue. After rotating the homogenate for 1 h at 4°C, the samples were centrifuged at 15,000 g during 10 min at 4°C and the supernatant was recovered. Protein concentrations were subsequently determined using the Bradford technique. Thirty (*Study III*) or 40 (*Study I and II*) μ g of protein per sample were loaded on 7.5 or 10% SDS precast gels (Bio-Rad, Hercules, California, USA) and separated through electrophoresis together with a protein ladder. Gels were subsequently transferred to PVDF membranes, using either a wet transfer of 90 min at 90 V (*Study I and II*), or the Trans-Blot Turbo Transfer System from Bio-Rad (*Study III*). Blocking was completed using fluorescent blocking buffer (Millipore, Billerica, MA, USA) during 60 min at room temperature (RT). Membranes were incubated overnight at 4°C with phospho-specific primary antibodies for mTOR (Ser2448, 1:500), p70S6K (Thr389, 1:1,000), eEF2 (Thr56, 1:1,500), rpS6 (Ser235/236, 1:1,000), 4E-BP1 (Thr37/46, 1:1,000) and AMPK α (Thr172, 1:1,000). All antibodies were from Cell Signaling Technology (Beverly, MA, USA), except for the antibody against p70S6K, which was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). After the overnight incubation, membranes were washed (4 x 5 min) in PBST (0.1%) and incubated with IRDye secondary antibody (LI-COR Biosciences, Cambridge, UK) for 60 min at RT. A final series of washes were then performed before the membranes were scanned using Odyssey SA Infrared Imaging System (LI-COR Biosciences). The blots were subsequently quantified using ImageJ, and phosphorylated proteins were expressed relative to total α -tubulin abundance (1:20,000; Sigma-Aldrich, St. Louis, MO, USA).

Immunohistochemical analysis

In *Study II*, a ~20 mg portion of each muscle biopsy was oriented for transverse sectioning and 5 μ m cross-sections were cut in a cryostat at -22°C and mounted on glass slides. Sections were stained using monoclonal antibodies to detect slow myosin heavy chain (A4.840 from Developmental Studies Hybridoma Bank, Iowa City, Iowa, USA), cell borders (Merosin IgG1, Novocastra Laboratories Ltd, Newcastle, UK), capillaries (LamA IgG2a, Novocastra) and myonuclei (DAPI, Novocastra). The majority of each biopsy section was captured (5–7 images) in x20 magnification (DFC360 FX Leica Microsystems, Wetzlar, Germany). To determine the frequency of fibres expressing slow myosin heavy chain (type I fibres), and to measure fibre CSA using custom-developed semi-automated analysis software, an average of 177 fibres (range 101 to 250) from each sample were analysed. The same

sections were used when counting the number of capillaries, which were related to the number of fibres and to fibre CSA. Muscle fibre myonuclei (sublaminar placement) were counted in three randomly selected areas (~100 fibres) per biopsy, and related to the number of fibres and to fibre CSA.

Protein concentration

The protein concentrations of different sub-fractions (total/mixed, sarcoplasmic and myofibrillar) were determined from samples obtained at rest in *Study II*. About 15 mg of muscle tissue was manually homogenised in RIPA buffer. The resulting homogenate was rotated gently for 60 min at 4°C. While 20 µl was used to measure the mixed/total protein concentration, the remaining portion was centrifuged at 20,000 g for 30 min at 4°C. The supernatant was recovered and taken as the sarcoplasmic fraction. The pellet was resuspended in a sucrose buffer with NaOH, then manually homogenised and boiled at 37°C for 60 min. The resulting homogenate was taken as the myofibrillar fraction. Protein concentrations were determined through the Bradford technique with BSA as standard.

Enzyme and glycogen assays

Freeze-dried muscle samples (2–3 mg) were homogenised in phosphate buffer with 0.5% BSA. CS and phosphofructokinase (PFK) activity was subsequently determined by NAD⁺–NADH coupled reactions using fluorometric techniques [135]. Lactate dehydrogenase (LDH) content was assessed through spectrophotometry. Glycogen was hydrolysed enzymatically to free glucose and assayed by fluorometry. All enzymatic assays were run in duplicates.

Data analysis

Most dependent variables were analysed by two-way repeated measures ANOVA (factors time and leg). Variables that were compared in the basal state (e.g. fibre size, enzyme levels and glycogen content) were analysed by a one-way repeated measures ANOVA. Pearson's product moment correlation was used to examine the relationships between changes in acute molecular responses (p70S6K, myostatin and trunc-PGC-1 α) and training adaptations (muscle volume, fibre size and peak torque). Data normality was assessed through histograms and the Shapiro-Wilk test. If deemed necessary, data were log-transformed. Significant interactions were further examined with simple effect tests and the false discovery rate procedure was employed after a priori planned pairwise comparisons [55]. The level of significance was set at 5% ($P < 0.05$). All statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA). Data are presented as means \pm standard deviation (SD).

RESULTS

Acute exercise responses

Responses to acute AE are summarised in **Table 2**. In *Study II*, average power was 35% greater in the trained compared with the untrained state. RE performance was similar across legs in *Study I and II* (**Table 3**). However, in *Study III*, the AE+RE leg showed 10% lower ($P < 0.05$) peak power than the RE leg.

Table 2. Responses to the acute aerobic exercise bouts

	Study I AE-360-RE	Study II AE-360-RE		Study III AE-15-RE
		Untrained	Trained	
Average power (W)	38±8	37±5	50±11	37±6
Average HR (bpm)	124±17	122±13	137±14	118±18
HR at exercise completion (bpm)	164±16	164±18	171±17	161±14
Average blood lactate (mmol/l)	3.1±1.1	3.5±0.5	5.3±0.9	-
Blood lactate 1 min post (mmol/l)	4.6±1.4	5.0±1.1	7.2±2.6	-
Blood lactate 3 min post (mmol/l)	4.5±1.4	5.0±1.2	7.3±2.6	-
RPE at 40 min (central; 6–20 units)	14±1	14±2	15±1	13±2
RPE at 40 min (local; 6–20 units)	17±1	17±2	17±1	15±1
RPE at completion (central; 6–20 units)	17±2	16±2	17±1	14±3
RPE at completion (local; 6–20 units)	20±0	19±1	20±1	18±1

Note: Values are mean ± SD. No statistical comparisons were made. HR = heart rate; RPE = rating of perceived exertion

Table 3. Responses to the acute resistance exercise bouts

			Knee extension peak power (W)	Knee extension CON power (W)	Knee extension ECC power (W)
Study I		AE-360-RE	350±63	345±64	356±63
		RE	357±53	346±52	368±55
Study II	Untrained	AE-360-RE	361±122	347±113	375±131
		RE	372±113	355±105	389±122
	Trained	AE-360-RE	464±126	451±123	478±130
		RE	475±131	460±128	489±133
Study III		AE-15-RE	351±97*	348±101*	354±95*
		RE	387±88	382±88	391±90

Note: Values are mean ± SD. * Different ($P < 0.05$) from RE. CON = concentric; ECC = eccentric

Aerobic exercise training

Responses to AE training are outlined in **Table 4**. Marked improvements in training performance were seen over the 5 weeks. In *Study II*, average power increased 32% from the first to the last session. In *Study III*, a 36% improvement was noted.

Endurance performance

In *Study II*, both legs showed improved endurance (time to exhaustion) after training, yet the increase was more prominent (time x leg interaction, $P = 0.05$) after AE+RE than RE (**Table 5**). In *Study III*, time to exhaustion increased ($P < 0.05$) after AE+RE and was unchanged after RE.

Table 4. Responses to the aerobic training sessions

	Study II	Study III
	AE-360-RE	AE-15-RE
Average power (W)	43±8	46±13
Average power session 1 (W)	37±5	39±7
Average power session 15 (W)	49±10*	53±15*
Average HR (bpm)	124±14	120±17
HR at exercise completion (bpm)	163±16	160±18
RPE at 40 min (central; 6–20 units)	15±1	13±2
RPE at 40 min (local; 6–20 units)	17±1	16±1
RPE at completion (central; 6–20 units)	17±1	16±2
RPE at completion (local; 6–20 units)	20±0	19±1

Note: Values are mean ± SD. * Different ($P < 0.05$) from session 1. No other statistical comparisons were made. HR = heart rate; RPE = rating of perceived exertion

Table 5. Endurance performance in study II (AE-360-RE) and study III (AE-15-RE)

		AE+RE			RE		
		PRE	POST	Δ%	PRE	POST	Δ%
Time to exhaustion (s)	AE-360-RE ^{b, c}	590±104	752±129	29	553±98	659±109	19
	AE-15-RE ^{a, b, c}	632±105	770±145	22	624±151	654±142	5
W_{\max} (W)	AE-360-RE ^{b, c}	50±12	72±19	44	49±12	62±15	27
	AE-15-RE ^{a, b, c}	57±15	77±19	35	56±21	62±18	11
Peak HR (bpm)	AE-360-RE ^{b, c}	158±18	169±12	7	153±17	161±17	5
	AE-15-RE ^b	163±7	168±16	3	160±10	159±15	-1

Note: Values are mean ± SD. Significant effects ($P < 0.05$): a = interaction; b = leg; c = time. HR = heart rate; W_{\max} = maximal workload

Resistance exercise training

In both *Study II* and *III*, peak power rose almost linearly during the 12 RE sessions (Fig. 5). The increase from the 1st to the 12th session was ~25% for either leg. However, in *Study III*, the leg subjected to AE+RE performed ~20% lower ($P < 0.05$) peak power compared with RE across the 5-week training.

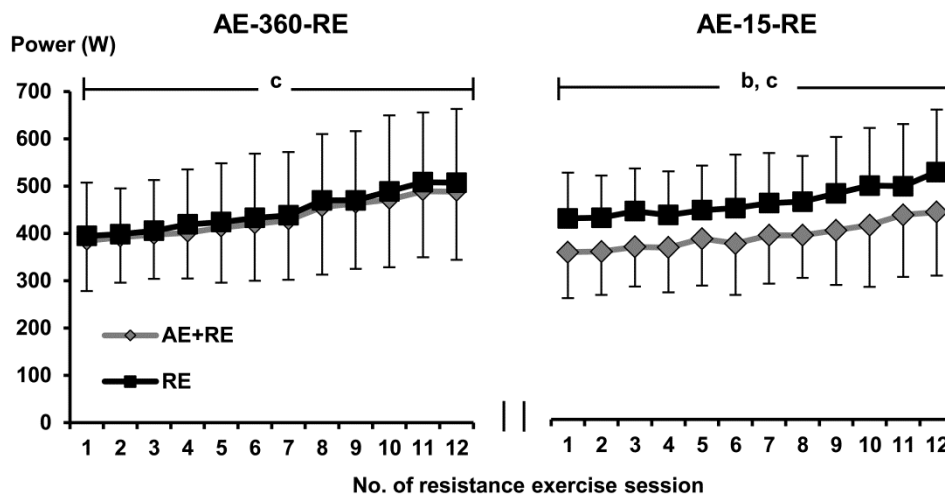


Figure 5. Peak power measured during each resistance training session in study II (AE-360-RE) and study III (AE-15-RE). Values are mean \pm SD. Significant effects ($P < 0.05$): b = leg; c = time.

Strength and power performance

In *Study II*, flywheel peak torque showed comparable increases after AE+RE and RE. Normalised torque showed a trend ($P = 0.08$) towards a time \times leg interaction (Table 6), due to a greater increase for RE (19%) than AE+RE (12%). In *Study III*, flywheel peak torque also increased after training. However, while peak eccentric torque increased equally across legs, peak concentric torque increased after RE (10%, $P < 0.05$), but not after AE+RE. Hence, concentric torque normalised to muscle CSA was compromised by the AE+RE intervention (interaction $P < 0.05$).

Table 6. Strength and power performance in study II (AE-360-RE) and study III (AE-15-RE)

		AE+RE			RE		
		PRE	POST	Δ%	PRE	POST	Δ%
Flywheel peak power (W)	AE-360-RE ^c	400±137	514±129	29	406±120	502±126	24
	AE-15-RE ^c	428±110	496±129	16	435±80	515±109	18
Flywheel peak torque (Nm)	AE-360-RE ^c	218±31	279±59	28	217±35	280±61	29
	AE-15-RE ^c	249±37	274±50	10	251±34	280±39	12
Flywheel CON torque (Nm)	AE-360-RE ^c	196±28	251±53	28	193±30	251±58	30
	AE-15-RE ^{a, c}	236±33	247±43	5	234±26	258±35	10
Flywheel ECC torque (Nm)	AE-360-RE ^c	240±36	307±66	28	242±42	308±68	27
	AE-15-RE ^c	263±44	301±63	14	268±46	303±51	13
Norm. torque (Nm/cm ²)	AE-360-RE ^c	2.77±0.37	3.10±0.52	12	2.81±0.36	3.36±0.69	19
	AE-15-RE	3.12±0.40	3.23±0.47	4	3.08±0.37	3.36±0.51	9
Norm. CON torque (Nm/cm ²)	AE-360-RE ^c	2.46±0.36	2.75±0.47	12	2.47±0.33	2.98±0.63	21
	AE-15-RE ^a	2.95±0.28	2.90±0.34	-2	2.87±0.26	3.08±0.39	7
Norm. ECC torque (Nm/cm ²)	AE-360-RE ^c	3.00±0.39	3.36±0.55	12	3.09±0.43	3.66±0.80	18
	AE-15-RE	3.29±0.55	3.56±0.66	8	3.29±0.54	3.64±0.71	11
Isometric peak torque (Nm)	AE-360-RE ^c	287±53	312±86	9	276±49	307±73	11
	AE-15-RE	323±84	305±52	-6	297±52	314±68	6
Isokinetic torque (Nm), 30 °/s	AE-360-RE ^c	248±35	278±71	12	227±52	263±54	16
	AE-15-RE	300±58	293±55	-2	291±46	296±56	2
Isokinetic torque (Nm), 60 °/s	AE-360-RE ^c	220±38	241±62	10	201±44	230±54	14
	AE-15-RE	265±57	254±46	-4	250±45	254±54	2
Isokinetic torque (Nm), 120 °/s	AE-360-RE ^c	198±48	211±62	7	176±32	193±39	10
	AE-15-RE	218±51	201±44	-8	204±35	206±49	2
Isokinetic torque (Nm), 180 °/s	AE-360-RE	164±37	183±62	12	151±37	164±40	9
	AE-15-RE	178±35	168±37	-6	173±29	173±41	0
Isokinetic torque (Nm), 210 °/s	AE-360-RE ^c	142±30	167±58	18	132±28	148±35	12
	AE-15-RE	164±39	148±28	-10	156±30	156±40	0
Isokinetic torque (Nm), 270 °/s	AE-360-RE ^c	118±33	151±56	28	122±25	130±33	7
	AE-15-RE	140±35	128±31	-9	140±26	141±37	1

Note: Values are mean ± SD. Significant effects ($P < 0.05$): a = interaction; b = leg; c = time. CON = concentric; ECC = eccentric; Norm. = normalised

Muscle hypertrophy and signal intensity

In *Study II*, QF volume showed a time x leg interaction ($P < 0.05$). The increase in muscle volume averaged 14% after AE+RE and 8% after RE (**Table 7**). The signal intensity of both QF and VL was similar across legs before training, and increased after AE+RE but not RE (interaction: $P < 0.05$). In *Study III*, QF volume and CSA increased after either intervention (**Table 7**). However, the increase was greater (interaction: $P < 0.05$) after AE+RE (6%) than RE (3%). Signal intensity increased 7% after AE+RE ($P < 0.05$), yet was unaltered by RE. The hypertrophy responses were very consistent across subjects (**Fig. 6**). The increased volume of the four individual QF muscles was greater after AE+RE than RE in both training studies.

Table 7. Selected MRI measures in study II (AE-360-RE) and study III (AE-15-RE)

		AE+RE			RE		
		PRE	POST	$\Delta\%$	PRE	POST	$\Delta\%$
m. QF volume (cm ³)	AE-360-RE ^{a, b, c}	1147±249	1303±276	14	1111±262	1198±273	8
	AE-15-RE ^{a, c}	1217±246	1286±247	6	1239±231	1273±237	3
m. VL (cm ³)	AE-360-RE ^{a, c}	361±80	421±95	17	360±97	387±102	8
	AE-15-RE ^{a, c}	403±95	425±95	6	411±96	421±96	2
m. VI (cm ³)	AE-360-RE ^{a, b, c}	362±107	402±124	11	338±98	359±104	6
	AE-15-RE ^{a, c}	376±77	389±79	3	390±67	395±71	1
m. VM (cm ³)	AE-360-RE ^{a, c}	294±66	329±69	12	282±75	300±76	6
	AE-15-RE ^{a, c}	322±70	336±69	4	318±64	325±64	2
m. RF (cm ³)	AE-360-RE ^{a, c}	91±22	115±29	26	89±25	104±29	16
	AE-15-RE ^{a, c}	117±26	135±28	15	120±33	131±34	9
m. QF CSA (cm ²)	AE-360-RE ^{a, b, c}	79±10	90±10	14	78±12	84±12	8
	AE-15-RE ^{a, c}	81±13	85±13	6	82±13	84±13	3
m. QF SI (MGV)	AE-360-RE ^{a, c}	52±7	58±8	12	52±7	52±9	0
	AE-15-RE ^a	48±6	52±8	7	47±6	47±6	0
m. VL SI (MGV)	AE-360-RE ^{a, b, c}	53±8	58±8	9	52±8	51±8	-2
	AE-15-RE ^a	47±5	51±6	8	47±4	47±5	0
m. BF SI (MGV)	AE-360-RE	40±7	41±6	2	41±10	40±10	-2
	AE-15-RE	41±6	42±6	1	40±4	40±5	1

Note: Values are mean ± SD. Significant effects ($P < 0.05$): a = interaction; b = leg; c = time. QF = quadriceps femoris; VL = vastus lateralis; VI = vastus intermedius; VM = vastus medialis; RF = rectus femoris; CSA = cross-sectional area; SI = signal intensity; MGV = mean grey value

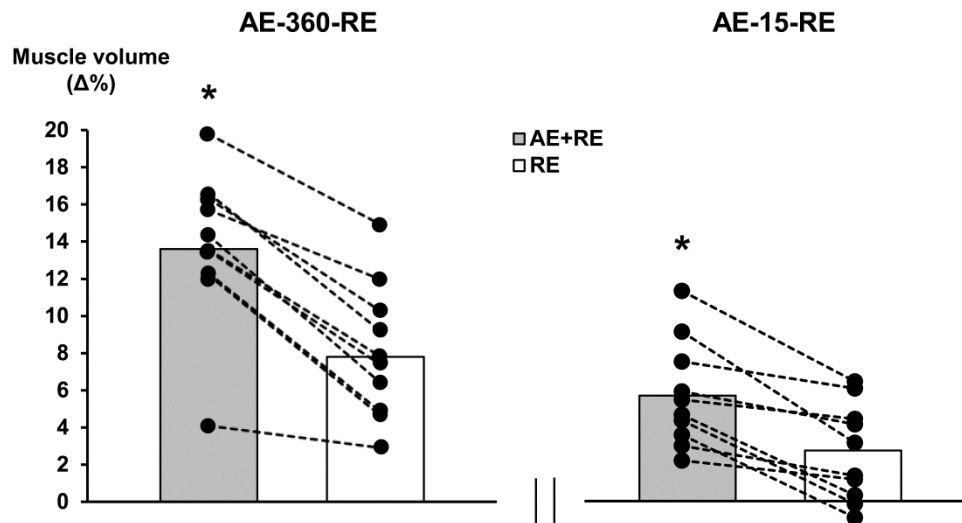


Figure 6. Relative changes in muscle size in study II (AE-360-RE) and study III (AE-15-RE).
* Different ($P < 0.05$) from RE.

Immunohistochemistry

In *Study II*, mean fibre CSA increased 17% following AE+RE ($P < 0.05$) compared with a 9% increase (non-significant) after RE (**Table 8**). Type II fibre CSA increased 19% after AE+RE ($P < 0.05$) and 16% after RE ($P = 0.05$). The number of nuclei per fibre did not change. Consequently, the average CSA covered by each nucleus increased after AE+RE ($P < 0.05$). The number of capillaries per fibre was unaltered by training, and hence the number of capillaries per CSA tended to decrease after AE+RE ($P = 0.07$).

Table 8. Immunohistochemical measures in study II (AE-360-RE)

	PRE	AE+RE	RE
Mean fiber CSA (μm^2)	4601 \pm 1097	5361 \pm 781*	5033 \pm 767
Type I fibre CSA (μm^2)	4055 \pm 911	4547 \pm 660	4259 \pm 932
Type II fibre CSA (μm^2)	5046 \pm 1439	5995 \pm 1010*	5829 \pm 999
Type I fibres (%)	50 \pm 15	51 \pm 18	51 \pm 13
Type II fibres (%)	50 \pm 15	49 \pm 18	49 \pm 13
Capillaries per fibre	2.38 \pm 0.61	2.37 \pm 0.68	2.51 \pm 0.62
Capillaries per mm^2	521 \pm 123	441 \pm 103	491 \pm 89
Nuclei per fibre	2.57 \pm 0.54	2.57 \pm 0.44	2.57 \pm 0.28
CSA (μm^2) per nuclei	1794 \pm 265	2089 \pm 237*	1959 \pm 261

Note: Values are mean \pm SD. * Different ($P < 0.05$) from PRE. CSA = cross-sectional area

Protein concentration

There were no differences (all $P > 0.05$) in protein concentrations across PRE vs. AE+RE vs. RE (analysed in *Study II* only). Mixed protein concentration was 134 ± 21 vs. 140 ± 27 vs. 145 ± 18 $\mu\text{g}/\text{mg}$ wet muscle respectively. These values were 86 ± 11 vs. 86 ± 12 vs. 86 ± 13 for the sarcoplasmic fraction and 60 ± 15 vs. 66 ± 18 vs. 60 ± 17 for the myofibrillar fraction.

Glycogen content

In *Study I*, glycogen decreased in response to exercise in both legs ($P < 0.05$; **Fig. 7**). However, glycogen content was overall lower in AE+RE compared with RE (main effect $P < 0.05$). In *Study III*, the leg that had completed AE showed 32% lower ($P < 0.05$) glycogen concentration than the rested leg. This effect was still evident 3 h after acute RE. Both the AE-360-RE and the AE-15-RE paradigms resulted in greater ($P < 0.05$) basal glycogen levels compared with RE (**Fig. 7**).

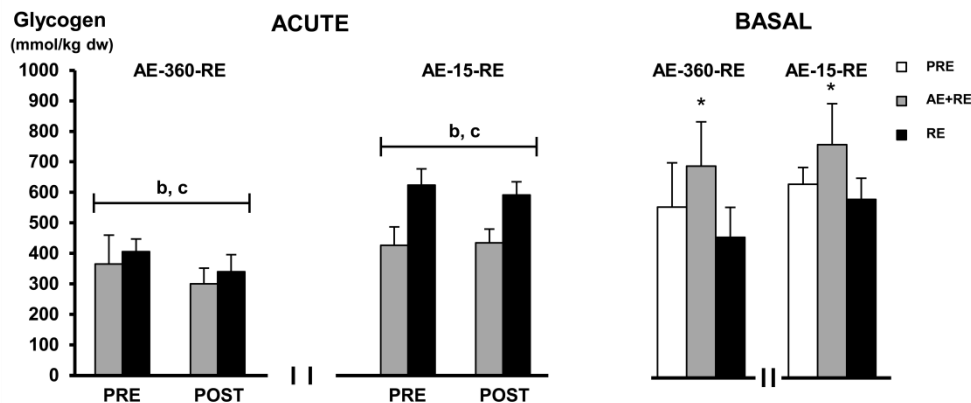


Figure 7. Left panel: glycogen concentrations before (PRE) and 3 h after (POST) acute resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise in study I/II (AE-360-RE) and study III (AE-15-RE). Right panel: basal glycogen levels after 5 weeks training of either regimen. Values are mean \pm SD. Significant effects ($P < 0.05$): b = leg; c = time. * Different ($P < 0.05$) from RE.

Enzyme activity

In *Study II*, CS activity increased 19% following AE+RE ($P < 0.05$), yet was unchanged after RE. Values were 34, 41 and 35 mmol/kg/min for PRE, AE+RE and RE respectively. PFK activity or LDH content showed no changes. In *Study III*, CS activity increased 18% after AE+RE ($P < 0.05$), yet remained unchanged after RE. Values were 45, 53 and 43 mmol/kg/min for PRE, AE+RE and RE.

Gene expression

Study I

There were time x leg interactions ($P < 0.05$) for both PGC-1 α and VEGF expression because mRNA levels were greater in AE+RE at PRE and POST1, compared with RE (**Fig. 8**). Expression of MuRF-1 over time was similar in AE+RE and RE (interaction $P = 0.08$). There was an interaction ($P < 0.05$) in atrogin-1 expression. Levels were higher in AE+RE at PRE and POST1 compared with RE ($P < 0.05$). There was also an interaction ($P < 0.05$) in myostatin expression. Levels were lower in AE+RE at PRE and POST1 compared with RE ($P < 0.05$). Within the RE leg, myostatin decreased ($P < 0.05$) ~60% from PRE to POST2 (**Fig. 8**).

Study II

Basal gene expression

There was no change in basal expression levels for any of the genes investigated.

Acute gene expression in trained compared with untrained muscle

In the untrained state, RE showed a 2-fold increase in PGC-1 α expression from PRE to POST ($P < 0.05$). This effect was offset in the trained state (**Fig. 9**). A condition x time interaction was also found ($P < 0.05$), due to decreased PGC-1 α expression in AE+RE from untrained to trained muscle, with no change in RE. VEGF expression increased after RE in the untrained but not trained state (**Fig. 9**). There was a condition x leg interaction ($P < 0.05$) for MuRF-1 expression due to a decrease in AE+RE with no change in RE (**Fig. 10**). In addition, MuRF-1 mRNA content was higher in AE+RE than RE in the untrained condition ($P < 0.05$). The opposite was true in the trained condition, i.e. expression levels were lower after AE+RE than RE ($P < 0.05$). AE+RE showed greater ($P < 0.05$) atrogin-1 expression than RE in the untrained condition (**Fig. 10**). In contrast, atrogin-1 expression was greater after RE than AE+RE in the trained condition ($P = 0.05$). Myostatin showed time x leg interaction ($P < 0.05$) in the trained state. Thus, myostatin levels decreased in RE from PRE to POST, while AE+RE showed no change (**Fig. 10**).

Isoform-specific PGC-1 α expression

There was a time x leg interaction ($P < 0.05$) for PGC-1 α -ex1a. Thus, while the expression decreased after AE+RE, it remained unchanged after RE (**Fig. 11**). There were also interactions ($P < 0.05$) in PGC-1 α -ex1b and non-trunc-PGC-1 α expression because values increased after RE, but not after AE+RE (**Fig. 11**). Trunc-PGC-1 α (untrained) showed no interaction. However, there was a main effect of time ($P < 0.05$) due to increased trunc-PGC-1 α levels from PRE to POST for both AE+RE (1.4-fold) and RE (2.2-fold).

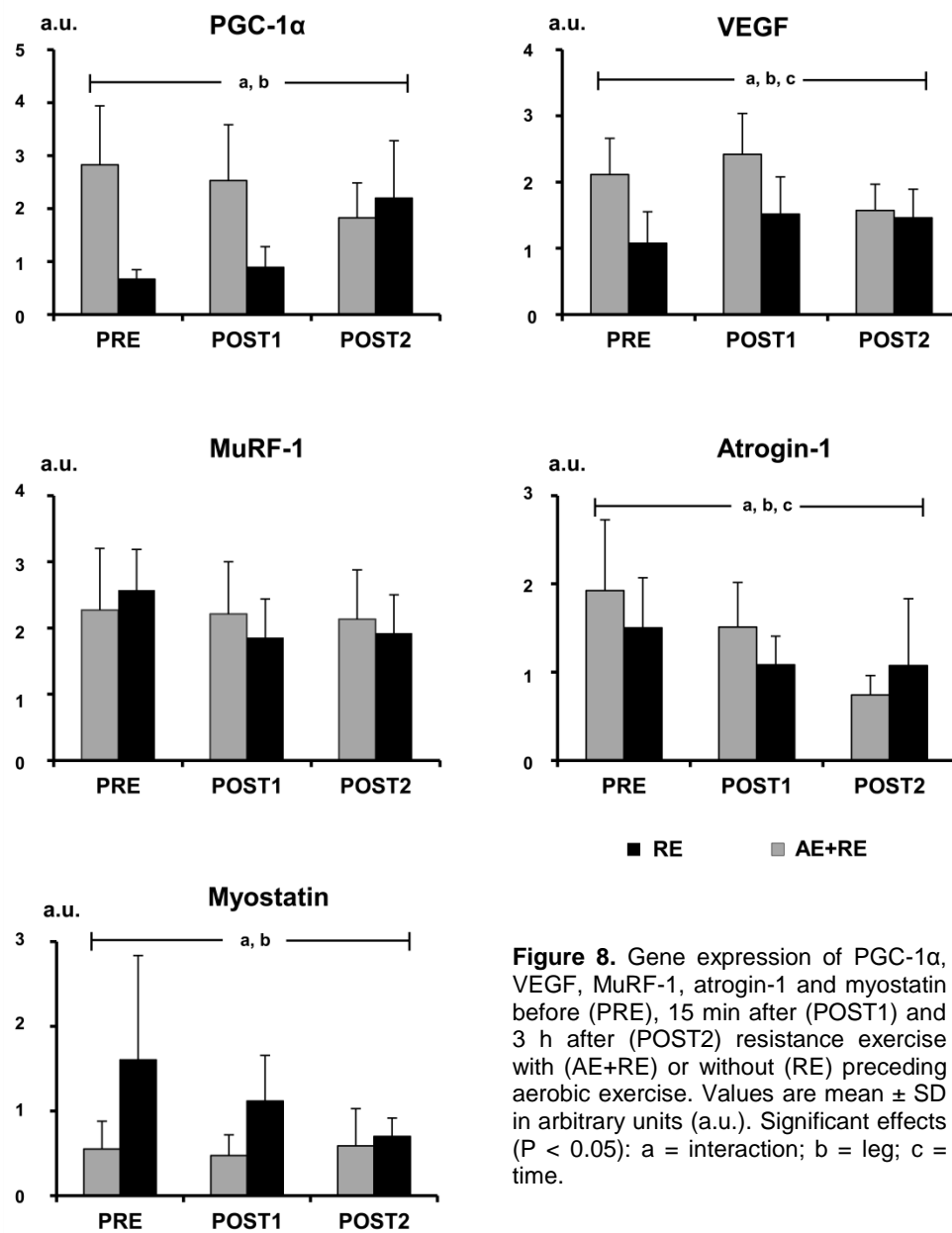


Figure 8. Gene expression of PGC-1 α , VEGF, MuRF-1, atrogin-1 and myostatin before (PRE), 15 min after (POST1) and 3 h after (POST2) resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise. Values are mean \pm SD in arbitrary units (a.u.). Significant effects ($P < 0.05$): a = interaction; b = leg; c = time.

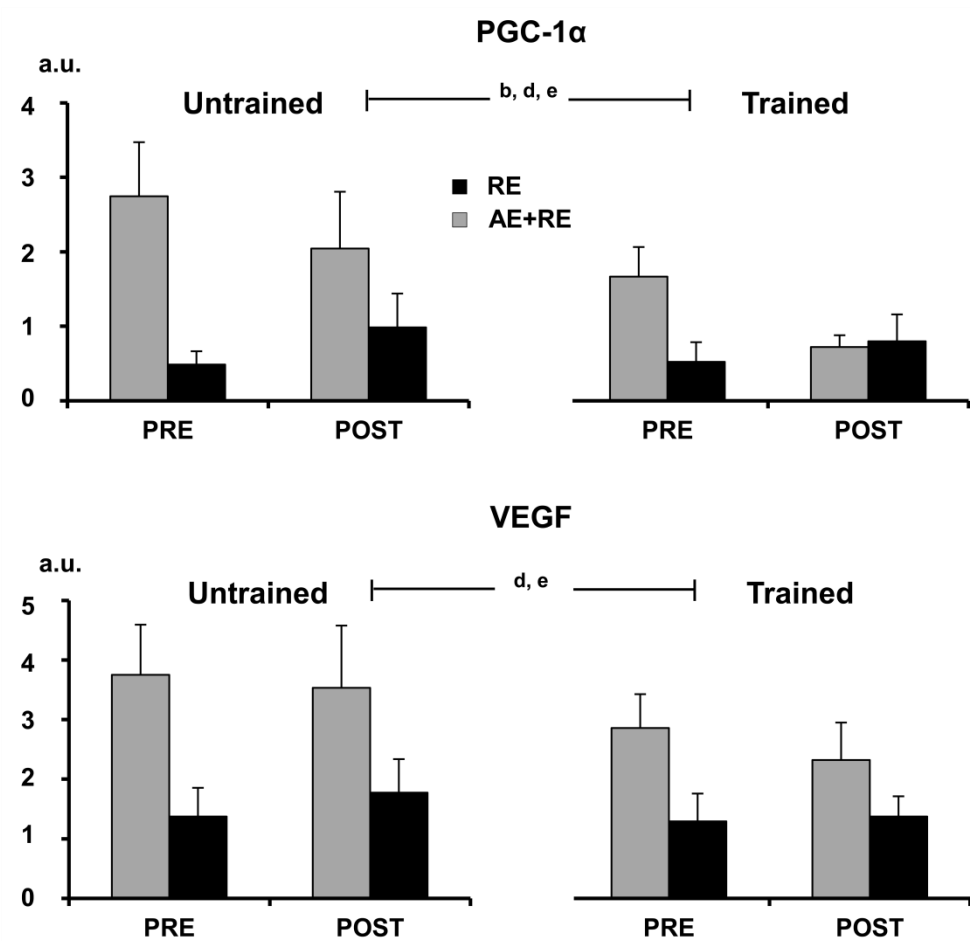


Figure 9. Gene expression of PGC-1 α and VEGF before (PRE) and 3 h after (POST) resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise in the untrained and trained condition. Values are mean \pm SD in arbitrary units (a.u.). Significant effects ($P < 0.05$): b = condition \times leg interaction; d = leg; e = condition.

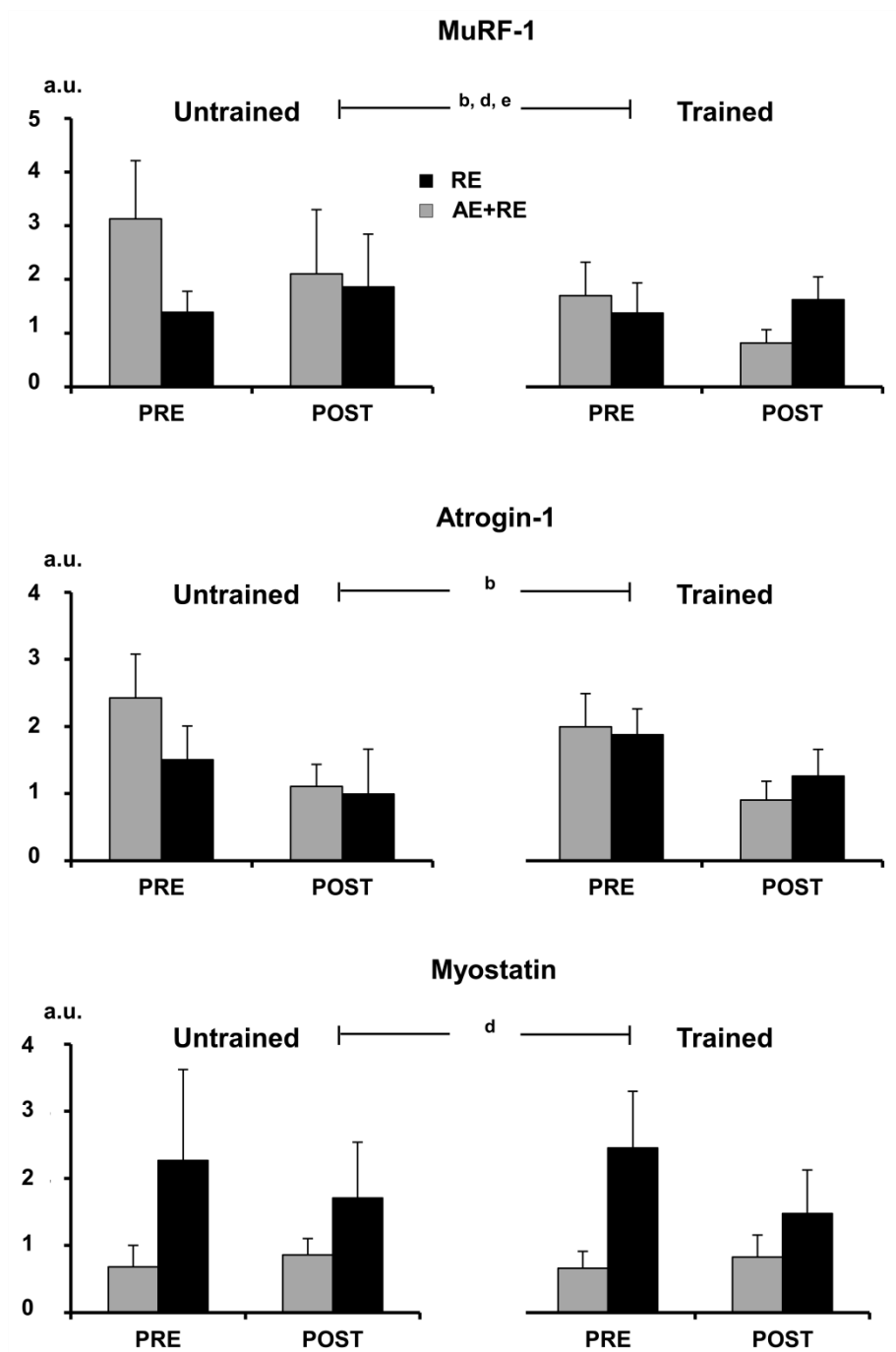
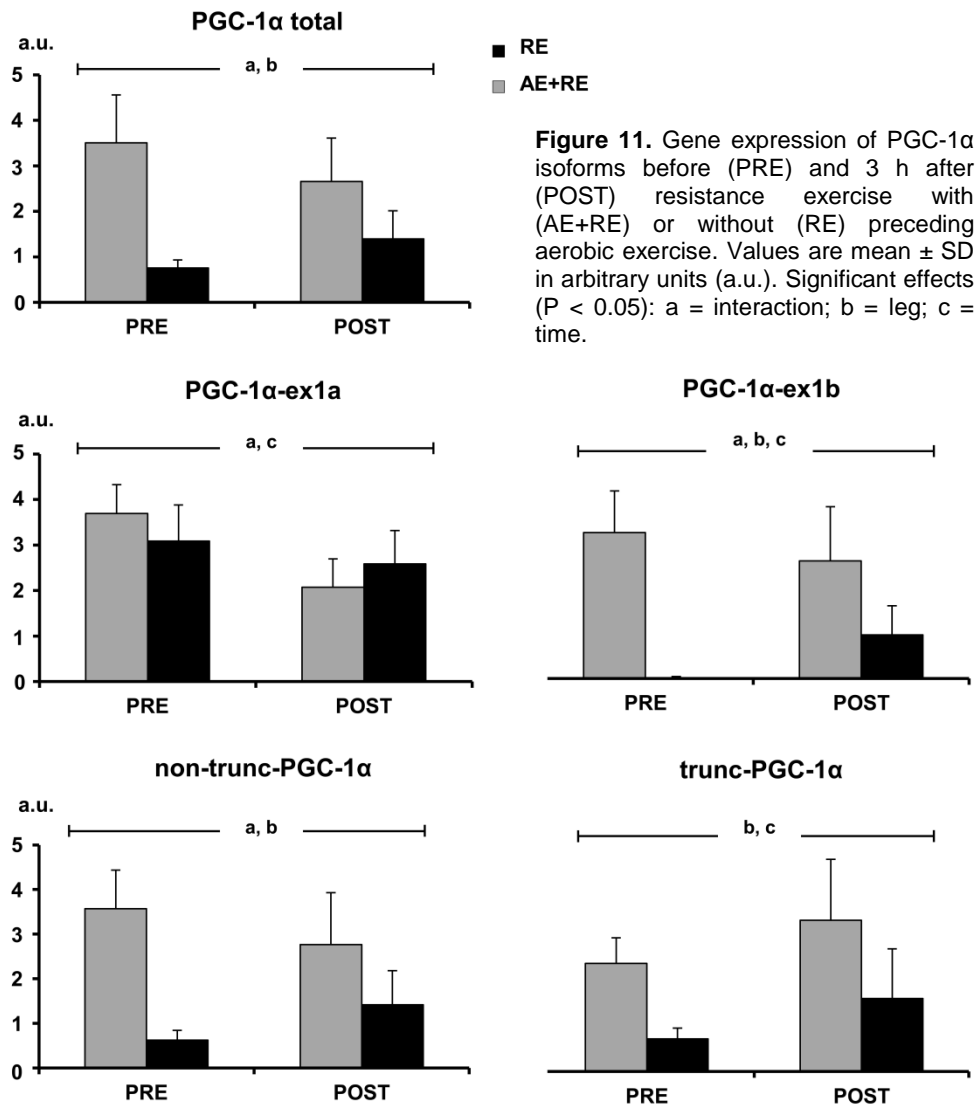


Figure 10. Gene expression of MuRF-1, atrogin-1 and myostatin before (PRE) and 3 h after (POST) resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise in the untrained and trained condition. Values are mean \pm SD in arbitrary units (a.u.). Significant effects ($P < 0.05$): b = condition x leg interaction; d = leg; e = condition.



Study III

There were time \times leg interactions for PGC-1α and VEGF expression ($P < 0.05$). Thus, the increased expression from PRE to POST was greater after AE+RE than RE (Fig. 12). MuRF-1 expression increased after AE+RE and was unchanged after RE (interaction: $P < 0.05$). Atrogin-1 showed interaction ($P < 0.05$), as mRNA-levels decreased after RE and tended to increase after AE+RE. Myostatin expression also showed an interaction effect ($P = 0.05$) because the down-regulation was greater after AE+RE than RE (Fig. 12).

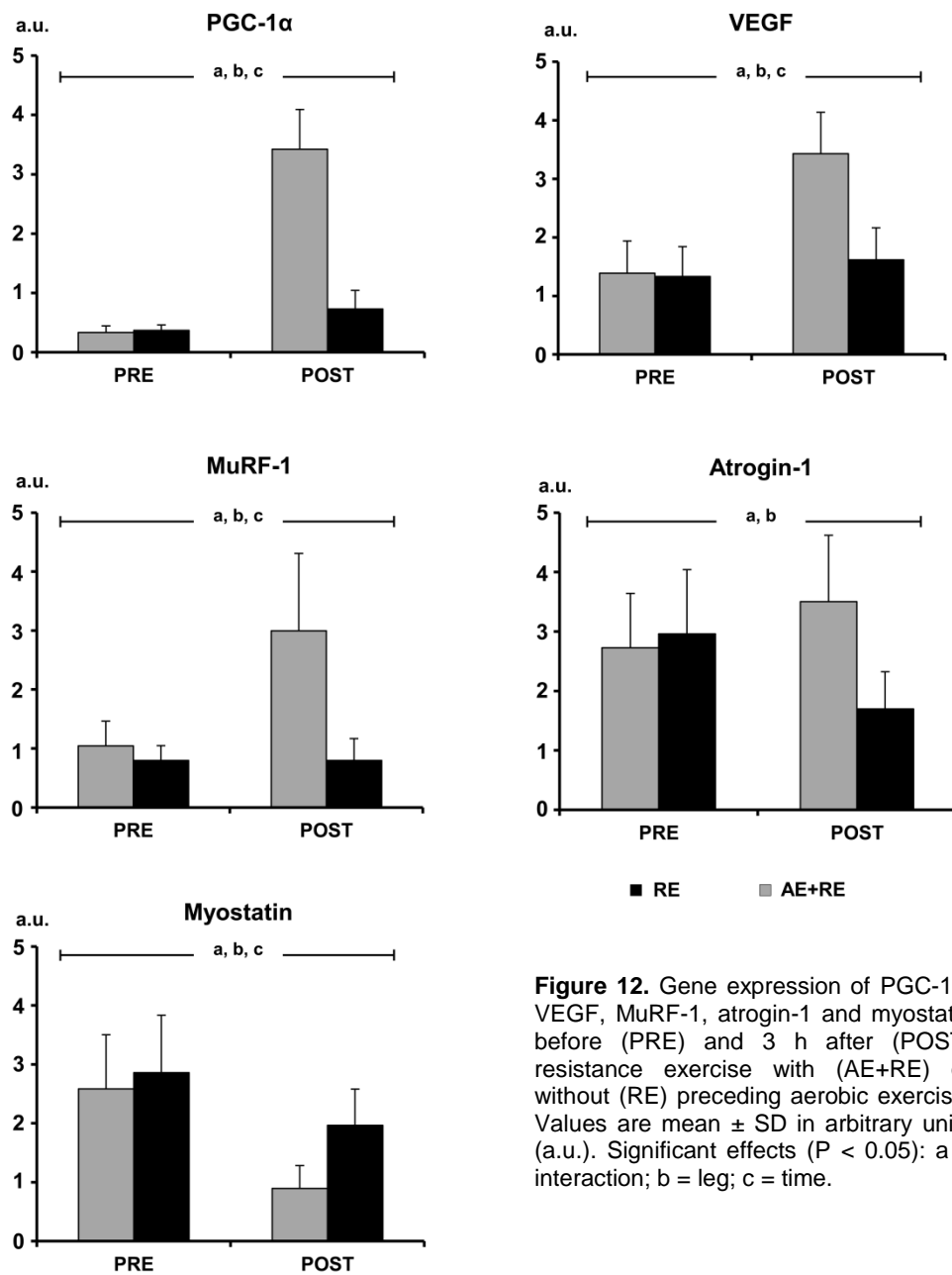


Figure 12. Gene expression of PGC-1α, VEGF, MuRF-1, atrogin-1 and myostatin before (PRE) and 3 h after (POST) resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise. Values are mean \pm SD in arbitrary units (a.u.). Significant effects ($P < 0.05$): a = interaction; b = leg; c = time.

Protein phosphorylation

Study I

There was no time x leg interaction in mTOR phosphorylation (**Fig. 13**). However, signalling was greater in AE+RE compared with RE ($P < 0.05$). Phosphorylation of p70S6K was also greater in AE+RE compared with RE ($P < 0.05$). Phosphorylation of rpS6 and eEF2 remained unchanged (data not shown).

Study II

There was a tendency towards a time x leg interaction ($P = 0.08$) for p70S6K in the untrained state (**Fig. 14**). Thus, while both legs showed increased p70S6K phosphorylation, the increase was slightly greater in AE+RE than RE (1.6 vs. 1.3-fold). In the trained state, both AE+RE and RE showed increased p70S6K phosphorylation. Overall p70S6K phosphorylation levels were greater in the trained compared with the untrained state (**Fig. 14**, main effect $P < 0.05$). Phosphorylation of mTOR, rpS6 and eEF2 was unchanged (data not shown).

Study III

AMPK phosphorylation showed a time x leg interaction ($P < 0.05$); values at PRE were greater (1.5-fold, $P < 0.05$) in the leg that had performed AE, compared with the rested (RE) leg (**Fig. 15**). Similarly, rpS6 showed interaction ($P < 0.05$) such that phosphorylation was elevated (1.3-fold, $P < 0.05$) in AE+RE compared with RE at PRE (**Fig. 15**). Phosphorylation of 4E-BP1 and p70S6K was unaltered (data not shown).

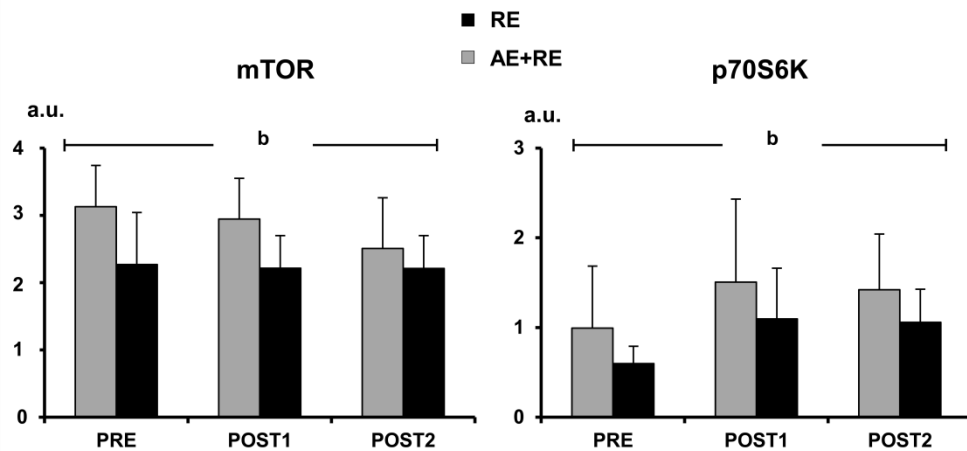


Figure 13. Phosphorylation of mTOR (Ser2448) and p70S6K (Thr389) before (PRE), 15 min after (POST1) and 3 h after (POST2) resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise. Values are mean \pm SD in arbitrary units (a.u.). Significant effects ($P < 0.05$): b = leg.

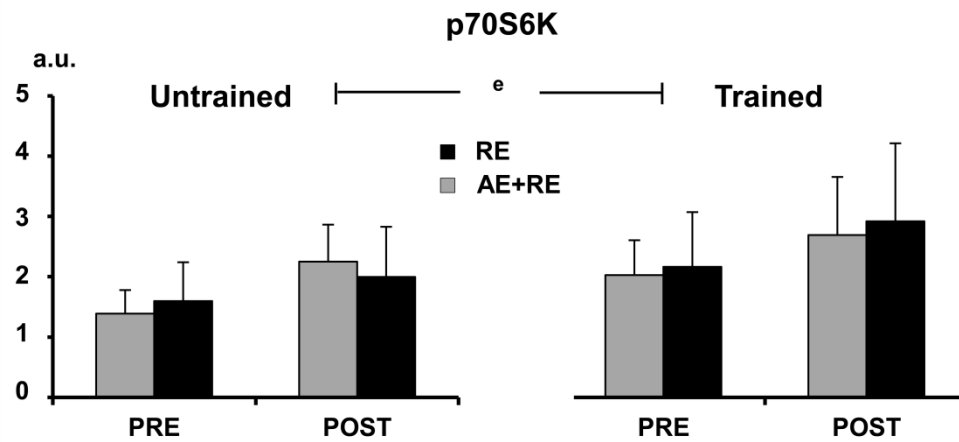


Figure 14. Phosphorylation of p70S6K (Thr389) before (PRE) and 3 h after (POST) resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise in the untrained and trained condition. Values are mean \pm SD in arbitrary units (a.u.). Significant effects ($P < 0.05$): e = condition.

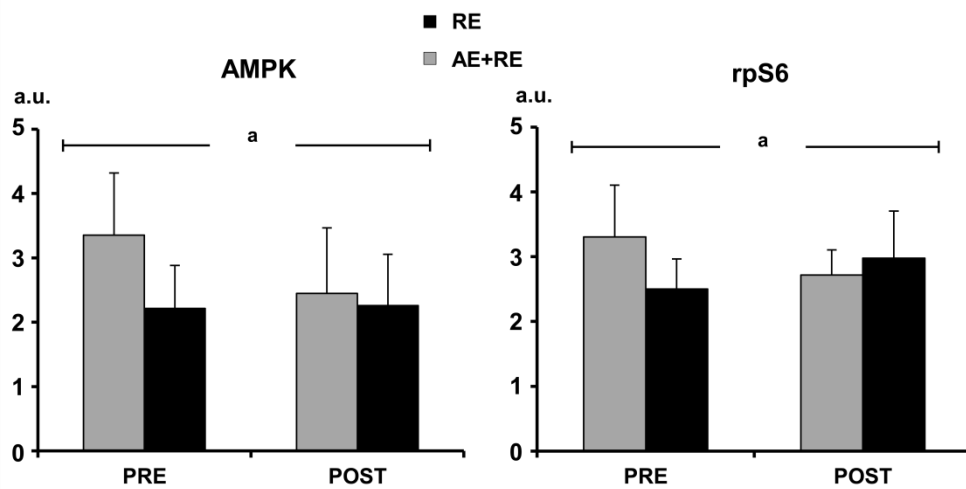


Figure 15. Phosphorylation of AMPK α (Thr172) and rpS6 (Ser235/236) before (PRE) and 3 h after (POST) resistance exercise with (AE+RE) or without (RE) preceding aerobic exercise. Values are mean \pm SD in arbitrary units (a.u.). Significant effects ($P < 0.05$): a = interaction.

Relationships between acute responses and training outcomes

There were no significant correlations between changes in selected molecular markers (p70S6K, myostatin and trunc-PGC-1 α) after acute resistance exercise, and training-induced adaptations (muscle volume, fibre size and peak torque; all $R < 0.5$ and all $P > 0.05$).

DISCUSSION

The interest in studying concurrent exercise arose from observations inferring that aerobic exercise (AE) could compromise established skeletal muscle responses prompted by resistance exercise (RE). It was hypothesised that acute AE would interfere with molecular responses to subsequent RE, such that increases in muscle size and strength to cumulative RE training would be compromised. As it turned out, and at surprise, AE+RE produced greater muscle hypertrophy than RE. In parallel, muscle anabolic environment was enhanced, and more so following AE+RE than RE. Thus, if anything, muscle growth responses to subsequent RE were potentiated rather than compromised by preceding AE.

A novel aspect of the study was that muscle molecular responses were examined in both the untrained and trained state. In supporting the whole-muscle adaptations demonstrated, molecular responses inferred more substantial tissue remodelling after AE+RE than RE of untrained muscle. Interestingly, responses in trained muscle were attenuated and more refined towards the specific exercise stimulus applied. These findings suggest that whole-muscle adaptations ultimately diverge in the direction predicted by the specific training stimulus, and further that aerobic exercise boosts muscle hypertrophy during short-term training. As no molecular marker correlated with training-induced changes in muscle size or strength, the results of the current exhaustive experiments offer no support to the idea that e.g. post-exercise gene expression or protein phosphorylation predict chronic exercise outcome.

When 6 h recovery was employed between exercises, peak power had returned to baseline and strength and power progressed at identical rates across AE+RE and RE. Conversely, incomplete recovery prompted reduced training performance and blunted concentric strength development compared with RE alone. Thus, imperatively, allowing for normalisation of muscle function between exercise bouts is crucial for optimal gains in strength and power.

Study design and the unilateral exercise model

The one-legged exercise model, targeting an isolated muscle group, allowed for strictly controlled intra-subject comparisons. The aerobic stimulus offers an intense and prolonged challenge at the muscle cell level. Oxygen uptake rises linearly with increased external work, and at maximal work rate, metabolic rate of active muscles increases more than 100-fold [10]. During the ~45 min exercise performed in the current study, ~2,500 concentric muscle actions were executed at a work rate of $\geq 70\% W_{\max}$. Thus, while this exercise modality does not resemble more familiar and established whole-body endurance tasks, the aerobic stress at the muscle

cellular level is undoubtedly challenging. This is justified by the findings of parallel increases in work capacity and oxidative enzyme activity reported here and in past research, employing one-legged cycle ergometry [118, 119].

The flywheel RE, conducted in the current series of experiments, offers unlimited resistance by means of coupled concentric-eccentric actions while providing brief eccentric overload. As shown here and elsewhere [162, 195], short-term training interventions using this approach result in robust muscle hypertrophy. Collectively, the paradigm, which aims at examining quadriceps-specific muscle adaptations to concurrent AE+RE, is believed to mimic conditions experienced in real-life sports training.

To assess the effects of AE on responses to RE, total work performed was markedly greater in the leg subjected to AE+RE. Yet, as both legs performed RE, it is unlikely cross-education effects biased the results [68, 148, 154]. Exercise in the current experiments was performed in the fed rather than the fasted state to ensure that any potential suppression of anabolic signalling would not be evoked by lack of nutrients [45].

Molecular responses to acute concurrent exercise

Enhanced translational capacity mediates exercise-induced increases in muscle protein synthesis. While mTOR and p70S6K phosphorylation was greater after AE+RE than RE (*Study I*), these differences were subtle and not always evident (*Study III*). Thus, although RE may elicit increased protein synthesis unaccompanied by detection of mTOR-signalling [59], augmented protein kinase activity alone cannot explain the more substantial muscle hypertrophy noted after 5 weeks AE+RE compared with RE training.

Myostatin is a negative regulator of muscle hypertrophy [44] and both AE and RE diminished myostatin expression in the current study. Previous studies are commensurate with that contractile activity, rather than any specific mode of exercise, stimulates down-regulation of myostatin [134, 139]. Hence, concerting the robust increase in whole-muscle and fibre size after AE+RE, prolonged myostatin suppression most likely favoured positive muscle protein turnover in the current study.

The ubiquitin ligase proteins MuRF-1 and atrogin-1 take part in regulating muscle protein turnover [26, 44]. Atrogin-1 expression either decreases [134, 139] or shows no change [47, 214] in response to RE. AE induces increased atrogin-1 expression in both trained [47] and moderately trained [134] subjects. MuRF-1 expression

increases following acute AE or RE [134, 214]. Yet, chronic training may attenuate this increase [139]. Given that MuRF-1 and atrogin-1 appear instrumental in governing load-induced muscle hypertrophy [54, 104], the up-regulation of these genes presumably augmented muscle growth in response to the current AE+RE paradigm.

Increased expression of PGC-1 α [161] and VEGF [83], reflecting mitochondrial biogenesis and angiogenesis respectively, occurs in response to AE. The increased PGC-1 α and VEGF mRNA after AE+RE is consistent with these findings. However, and in contrast to results indicating that PGC-1 α expression responds exclusively to endurance-type exercise [12, 47], it also increased 3 h after RE. This marked increase of PGC-1 α in response to no more than 28 forceful muscle actions was a secondary, yet intriguing, finding of *Study I* which spurred analysis of isoform-specific PGC-1 α expression (discussed later).

As the molecular response to acute exercise is influenced by genetic variability [201], and cross-talk, redundancy and transiency of activation [44], the particular time-point chosen to study the selected markers is critical. The 3 h post-RE time-point accommodated changes in both protein phosphorylation and gene expression. Thus, genes regulating protein turnover typically show responsiveness to acute RE within this time frame [134], accompanied by elevated phosphorylation of selected proteins [208].

In summary, the selected molecular targets represent key mediators of exercise-induced muscle adaptations. Their response aids in explaining why AE+RE served as a more potent stimulus for muscle hypertrophy than RE. More specifically, AE elicited responses not very different from those evoked by RE. This would suggest that the summative response of AE+RE was responsible for the greater hypertrophic response noted with this exercise paradigm.

Muscle hypertrophy in response to chronic concurrent exercise

Perhaps the most striking finding of the current thesis was the most impressive increase in muscle size following AE+RE. The increase in muscle size was very consistent and the hierarchical response across individual QF muscles was similar to what has been reported elsewhere employing this particular RE regimen [162, 195]. Moreover, signal intensity of the RE leg and the non-used BF was unaltered, providing further evidence that reported differences across legs were specific to QF muscles and the intervention imposed. Increases in whole-muscle and fibre CSA occurred in parallel (*Study II*). The more robust effect shown after AE+RE appeared to be ascribed to a more substantial type I fibre hypertrophy. Altogether

the results suggest that repetitive concentric low-force actions act synergistically with high-force RE to govern increased skeletal muscle size at a rate greater than shown with established highly effective RE training. While this contrasts with the general view that AE prompts no or minute muscle hypertrophy [123], it is worth noting that one-legged cycling performed 5 days/week for 5 weeks increased muscle size by 7% in recreationally active men [153], and low-force actions, performed with or without vascular occlusion, induced hypertrophy similar to traditional high-force RE training [9, 98, 150, 160]. Evidently, low-force actions repeated until contractile failure may represent a strategy to induce significant hypertrophy [33, 122], albeit not necessarily as powerful as more traditional high-loading regimens.

The exaggerated increase in size was accompanied by augmented signal intensity of MRI scans after AE+RE but not RE. Typically, exercise-induced increases in signal intensity are attributed to increased muscle water/hydrogen content, due to very transient osmotic fluid shifts [146], or as a result of oedema from muscle damage after e.g. eccentric exercise [72]. None of these explanations could account for the increased signal intensity because MRI scans were obtained at least 48 h after completing the final exercise session. At this time, both legs had performed the eccentric component of RE, unaccompanied by muscle soreness. However, as mitochondria may constitute 4–6% of muscle volume density [100] and proxy markers examined (CS activity; endurance performance) showed substantial increases after AE+RE, along with increased glycogen content, it is possible these constituents impacted muscle composition and hence signal intensity. Notwithstanding, resting samples obtained before and after training showed unaltered protein concentrations, to suggest that the increased muscle size was mainly due to contractile protein accretion.

It was hypothesised that the induction of antagonistic molecular signalling pathways would compromise muscle hypertrophy to AE+RE [46, 91]. Animal and *in vitro* studies suggest that AMPK signalling blocks mTOR activity [105], impairs protein synthesis [28, 199], and provokes myofibrillar protein degradation [157]. In contrast, the current data clearly show that hypertrophy was not compromised by AE+RE. This may be because AMPK is activated momentarily in response to exercise. Increased AMPK activity is typically evident ≤ 1 h post-exercise [36, 60], returning to baseline shortly thereafter [60, 208], and cumulative exercise may even offset this response [144]. Given that protein synthesis may be elevated 48–72 h after acute RE [125], the very brief AMPK activation induced by AE appears to evoke little, if any, impact on the net protein balance accumulated between exercise sessions. It is therefore concluded that in humans, AMPK activation induced by AE does not blunt hypertrophy in response to subsequent and cumulative RE.

AE reduces glycogen stores and commencing exercise in the low-glycogen state may alter metabolic and molecular responses compared with muscles displaying normal or high glycogen levels [51, 92]. The current study showed that glycogen depletion induced by AE was not accompanied by altered mTOR-signalling 3 h post-RE. Similarly, translational signalling and protein synthetic responses to RE were not compromised in glycogen-depleted skeletal muscle at onset of exercise [37]. Thus, human muscle commencing RE at ~30% reduced glycogen stores (*Study III*) clearly possesses undiminished ability to undergo hypertrophy.

Progression of *in vivo* muscle function

In *Study II*, day-by-day power and total work produced during each of the 12 RE sessions were almost identical across legs. Accordingly, increases in *in vivo* muscle strength and power as a result of training, were very similar across legs and hence exercise modes. Thus, when 6 h recovery was allowed between sessions, AE did not interfere with RE performance outcomes.

Conversely, when recovery was reduced to 15 min (*Study III*), AE impaired subsequent RE performance throughout the training period. Improvements in concentric strength after 5 weeks were greater with RE than with AE+RE. These findings are consistent with the view that AE interferes with RE-induced progression of muscle function [131], and perhaps most evident for explosive strength and power [62, 77, 147, 165], and not necessarily paralleled by compromised muscle hypertrophy [86, 107, 147]. For example, back-to-back AE+RE impeded progression of strength, but not muscle size [179], and high- but not low-intensity RE produced strength gains, whereas muscle size increased regardless of intensity [98]. Thus, given that interference in strength development is not always accompanied by reduced rate of muscle hypertrophy, it must be that neural factors play a role in the interference effect. While acute AE results in transient neuromuscular dysfunction [21, 120], the response to chronic AE has been less researched [86]. Notwithstanding, the current results suggest that restoring muscle function between exercise bouts is vital for attaining optimal gains in strength and power. Therefore, athletes and individuals aiming at developing these qualities should ensure sufficient recovery time between contrasting exercise modes.

Molecular responses in untrained and trained muscle

Clearly, chronic exercise training modifies skeletal muscle molecular responses to acute exercise [208]. Therefore, physiological extrapolations from responses brought about by acute exercise bouts could be misleading, if not erroneous. To

this end, molecular responses were assessed and compared in the untrained and trained state (*Paper III*). Chronic training augmented the acute p70S6K response. Similarly, rat muscle showed summated anabolic and attenuated catabolic signalling following repeated bouts of RE [113]. Thus, cumulative training may boost the acute p70S6K response, perhaps allowing for a more rapid translation initiation after exercise. This would be consistent with the shorter duration of, yet more pronounced initial increase in, muscle protein synthesis after acute RE, evident in the trained state [191].

MuRF-1 and atrogin-1 showed greater expression after acute AE+RE than RE in the untrained state. Given that both genes are involved in proteasome-dependent protein breakdown, an event necessary for exercise-induced tissue remodelling and muscle growth [54, 104, 128], it is likely the exacerbated response enabled more marked muscle hypertrophy following AE+RE. Interestingly however, responses were attenuated with chronic training and MuRF-1 was even lower in AE+RE compared with RE in the trained state. As it relates to initiating muscle hypertrophy, these novel results suggest that AE acts synergistically with RE only during short-term training.

The endurance markers (PGC-1 α and VEGF) responded to a novel RE bout in the untrained, but not the trained state. Moreover, exercise-induced expression of these genes was more short-lived in the trained condition, corroborating with the acute attenuation of PGC-1 α and VEGF typically seen with training [167, 176]. Hence, as global PGC-1 α expression after acute RE does not necessarily lead to enhanced muscle oxidative capacity or mitochondrial biogenesis after cumulative training, conclusions based on PGC-1 α following an acute exercise challenge, regarding chronic effects, should be exercised with caution.

Isoform-specific expression of PGC-1 α

In the process of work on this thesis, it was reported that a novel splice variant of PGC-1 α was preferentially induced by RE, and claimed to control muscle hypertrophy *in vivo* [177]. Interestingly, expression of this truncated splice variant termed PGC-1 α 4, was further augmented by concurrent AE training. Given that alternative mRNA splicing influences the co-activation of transcription factors regulating the physiological effect [218], it was proposed that isoform-specific induction of PGC-1 α would be the mechanistic link to the exaggerated muscle hypertrophy noted after concurrent exercise.

The current results inferred that expression of truncated and non-truncated isoforms increases after both AE and RE. Similarly, Ydfors et al. showed

comparable increases in trunc-PGC-1 α after single bouts of either exercise mode [215]. These acute data suggest that trunc-PGC-1 α does not respond differently to AE and RE respectively. With regard to chronic effects, no splice variant assessed in the current study showed a change in the basal state after training. As changes in basal levels of PGC-1 α transcripts are typically not present >24 h post-exercise [65, 121, 171], it is concluded that PGC-1 α isoforms are regulated transiently in response to acute exercise of any mode. Hence, the trumpeted role of PGC-1 α as a master regulator of exercise-induced hypertrophy [177] appears far-fetched.

Relationship between acute responses and training outcomes

If certain quantifiable acute events could explain the variance in training-induced muscle adaptations, they could serve as early and valid markers for e.g. the efficacy of various exercise programmes. Indeed, results have been posted to suggest that molecular markers could reflect muscle adaptive responsiveness to chronic RE training. Phosphorylation of p70S6K correlated with the subsequent increase in muscle mass following 6 weeks electrical stimulation in rats [15], and 14 weeks RE training in men [192]. Further, RE-induced down-regulation of myostatin correlated with increased 1RM strength and muscle size [174]. These are not consistent findings, and other reports have questioned the usefulness of sole molecular markers in explaining training outcomes [149, 150, 169].

In the current study, p70S6K phosphorylation explained no more than $\leq 20\%$ of the variance in muscle hypertrophy. At an individual level, this would have a most insignificant predictive value. Further, and at odds with a previous report [174], there was no correlation between changes in myostatin and whole-muscle or fibre size. Discrepancies in study results could be explained by multiple factors inherent in the study design, e.g. time of biopsy sampling, subjects' physical conditioning level and the particular exercise employed. Nevertheless, the results cast doubt on the hypothesis that acute changes in molecular snapshot markers could predict variability in muscle adaptations with any certainty. The heterogeneous response to exercise and the complex regulation of resulting phenotypic adaptations certainly limit the capability of single markers to explain muscle adaptability [4, 13, 201]. Thus, future studies should look beyond master regulators of specific muscle adaptations and perhaps explore global maps of transcriptional regulation [115] and miRNA expression [56].

Summary and conclusions

The main findings in this thesis were that:

- Concurrent exercise elicited greater mTOR and p70S6K phosphorylation compared with RE. In parallel, myostatin was suppressed for a longer time after AE+RE. These differences indicated that translational capacity was reinforced rather than compromised by AE+RE. Consequently, concurrent exercise appears to enhance skeletal muscle anabolic environment more than RE (*Paper I*).
- When employing 6 h recovery between exercise modes, muscle strength and power showed comparable improvements across legs, yet the increase in muscle size was more evident following AE+RE. Thus, AE could offer a synergistic hypertrophic stimulus to RE training without altering the progress in muscle function resulting from RE (*Paper II*).
- AE performed 6 h prior to RE augmented p70S6K signalling and MuRF-1/atrogen-1 expression in untrained muscle compared with RE alone. However these responses were blunted in the trained state, suggesting that AE acts synergistically with RE only during short-term training, as it relates to initiating muscle hypertrophy. Although the molecular adaptations at large were aligned with global muscle changes shown after 5 weeks training, no single molecular marker correlated with training-induced changes in muscle size, strength or power (*Paper III*).
- Both the truncated and non-truncated splice variants of PGC-1 α were expressed after AE and RE, respectively. Hence, these transcripts respond regardless of exercise mode. Furthermore, none of the splice variants were up-regulated in the rested state after training. Acute and chronic gene expression showed no correlation with muscle adaptations resulting from training. Thus, it appears highly unlikely that truncated PGC-1 α is a principal coordinator of human muscle hypertrophy (*Paper IV*).
- Activation of AMPK induced by AE did not compromise muscle hypertrophy in response to subsequent RE. However, performance of consecutive AE and RE bouts compromised concentric force development. Thus, while muscle hypertrophy appears unaffected by recovery strategy, restoring muscle function between exercise bouts is a prerequisite for maximal gains in strength and power (*Paper V*).

Perspectives

The results of the current investigation suggest that cellular responses induced by AE, generally held to be antagonistic to those elicited by RE, do not cause interference in strength and muscle mass development after short-term concurrent training. In fact, several cellular responses appear to be shared across exercise modes, indicating that contractile activity rather than specificity of exercise is the prime driver initiating early muscle-adaptive responses. However, in the trained state, molecular responses were attenuated and more specific to the exercise mode employed. Thus, with longer training periods (i.e. months to years), the stimulus imposed by AE is likely insufficient to promote hypertrophy. Also, as the molecular- and protein-synthetic response to acute exercise fades after chronic training, disturbances from antagonistic molecular signals may play a larger role in trained individuals possessing a smaller window to drive adaptive changes. This scenario (**Fig. 16**) could in part explain why it seems impossible to achieve the phenotype displayed by the extreme endurance athlete and the powerlifter at the same time.

In numerous sports, strength and power are prerequisites for performance, yet endurance is required as well. For those athletes, it appears skeletal muscle complies with intense RE and AE training over several weeks, perhaps months. In particular, this holds true if sufficient recovery is allowed between exercise modes and the overall training frequency, engaging the same muscles, is modest. It follows that individuals aiming at improving health, general fitness or body composition should not be too concerned about any negative impact of AE on strength and muscle mass. In fact, the combined training approach used in this study could be prescribed if a rapid gain in muscle mass is the prime objective. However, the serious athlete continuously needs to fine-tune key elements of training and hence programme design, and refrain from excess training stress/volume, to minimise the risk of incompatibility.

Given that concentric force was negatively impacted by aerobic training with no adverse effect on muscle size, it must be that neural adaptations play a part in any early interference effect. Future studies should address this issue in detail, along with additional systematic research quantifying potential inhibitory or stimulatory effects of AE on strength and power development resulting from RE training. Also, novel paradigms designed to optimise adaptations to concurrent training should be examined.

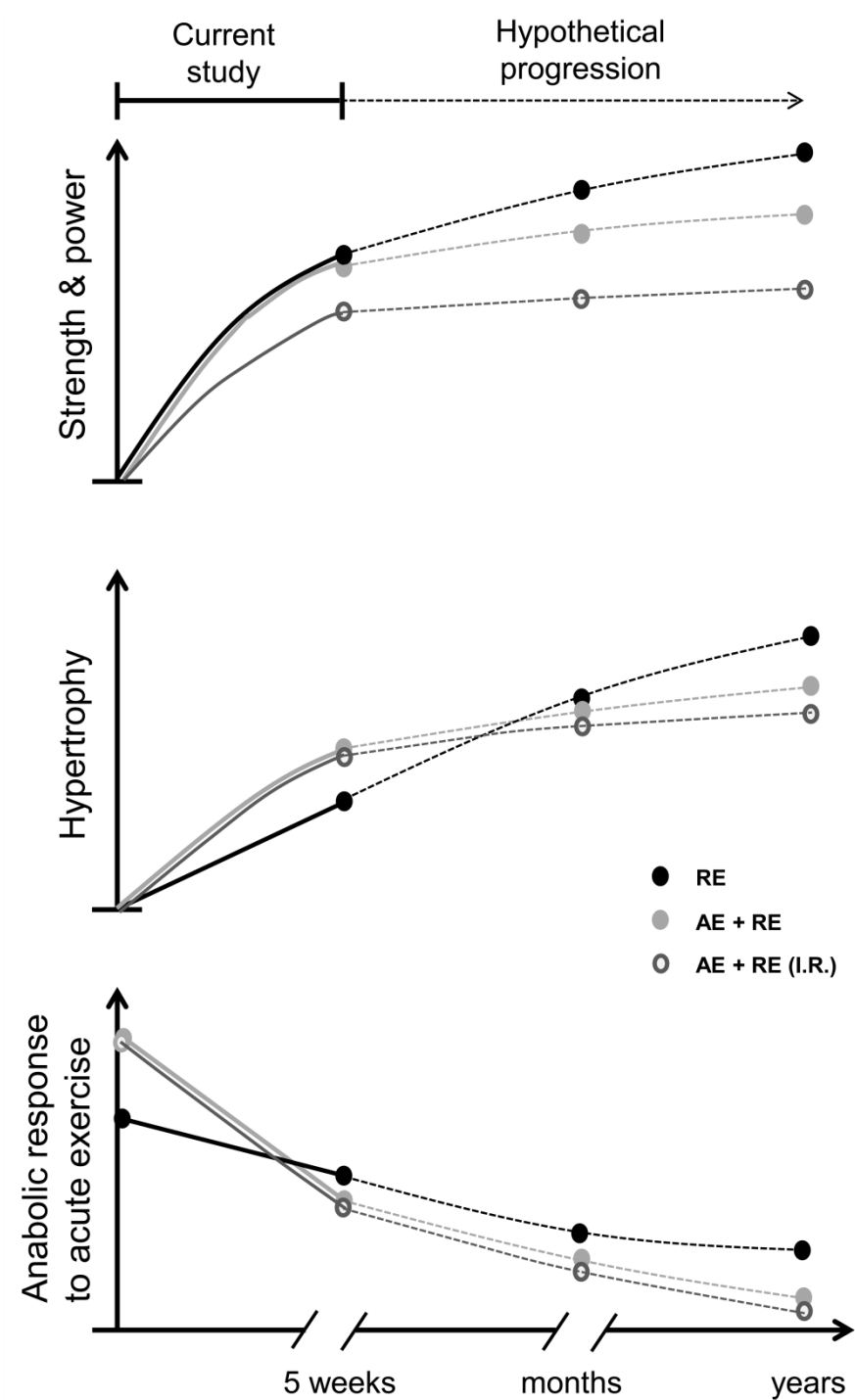


Figure 16. Hypothetical model describing adaptations to resistance (RE) training with or without preceding aerobic exercise (AE). See text for details. I.R. denotes incomplete recovery.

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