Title: Ribosome biogenesis adaptation and mTORC1 signalling in human skeletal muscle following concurrent training compared with resistance training alone.

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1. Key points summary

- Ribosome biogenesis is an important process linked with human skeletal muscle growth following resistance training (RT); however, whether concurrent training alters skeletal muscle ribosome biogenesis compared with RT alone in unknown
- In agreement with previous studies, concurrent training blunted the RT-induced increase in type I, but not type II, muscle fibre size
- Despite the attenuated muscle hypertrophy with concurrent training, changes in markers of skeletal muscle ribosome biogenesis were generally more favourable with concurrent training vs. RT performed alone
- Conversely, a single session of resistance exercise (RE) performed post-training was more potent for inducing signalling responses in skeletal muscle related to both ribosome biogenesis and the mTORC1 pathway, vs. concurrent exercise
- Ribosome biogenesis is therefore not compromised following short-term concurrent training; however, both mTORC1 and ribosome biogenesis-related signalling are attenuated in skeletal muscle following a single session of concurrent exercise performed in a training-accustomed state

2. Abstract

Combining RT with endurance training (i.e., concurrent training) may attenuate skeletal muscle hypertrophy consequent to RT; however, the underlying mechanisms are unclear. We investigated whether markers of ribosome biogenesis, a process linked with skeletal muscle hypertrophy, are attenuated following concurrent training vs. RT alone. Twenty-three males (mean ± SD: age, 29.6 ± 5.5 y; $\dot{V}O_{2peak}$, 44 ± 11 $mL \cdot kg^{-1} \cdot min^{-1}$) underwent 8 wk (3 $sessions \cdot wk^{-1}$) of either: 1) HIT (high-intensity interval training) combined with RT (HIT+RT group, *n*=8), 2) work-matched MICT (moderate-intensity continuous training) combined with RT (MICT+RT group, *n*=7), or 3) RT alone (RT group, *n*=8). *Vastus lateralis* biopsies were obtained before training, and immediately before, 1 h and 3 h after the final training session. Type I muscle fibre cross-sectional area (CSA) was further increased by RT vs. HIT+RT (34 ±22%; ES, 1.03 ±0.80), but not vs. MICT+RT (15 ±54%; ES, 0.39 ±1.45). Basal training-induced changes in expression of the 45S ribosomal RNA (rRNA) precursor, and 5.8S and 28S mature rRNAs were greater for concurrent exercise vs. RT, largely because of trends for reduced rRNA expression following RT. During the final training session, RT further increased skeletal muscle mTORC1 signalling (p70S6K1 and rps6 phosphorylation) and signalling related to 45S rRNA transcription (TIF-1A and UBF phosphorylation) vs. concurrent exercise. Thus, when performed in a training-accustomed state, RT preferentially induces mTORC1 and ribosome biogenesis-related signalling in human skeletal muscle vs. concurrent exercise. However, changes in markers of skeletal muscle ribosome biogenesis were more favourable with concurrent training vs. RT.

3. Abbreviations list

1-RM, one-repetition maximum; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; AMPK, 5' adenosine monophosphate-activated protein kinase; β2M, beta-2 microglobulin; CDK, cyclin-dependent kinase; DXA, dual-energy x-ray absorptiometry; Fox-O1, forkhead box-O1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIT, high-intensity interval training cycling; LT, lactate threshold; MICT, moderate-intensity continuous cycling; MPS, muscle protein synthesis; mRNA, messenger RNA; mTORC1, mechanistic target or rapamycin complex 1; MuRF-1, muscle RING-finger 1; p70S6K1, 70 kilodalton ribosomal protein subunit kinase 1; PGC-1α, peroxisome proliferator activated receptor gamma co-activator 1 alpha; POLR1B, polymerase (RNA) 1 polypeptide B; RE, resistance exercise; RPE, rating of perceived exertion; rRNA, ribosomal ribonucleic acid; RT, resistance training; SL-1, selectivity factor-1; TBP, TATA binding protein; TIF-1A, RRN3 polymerase 1 transcription factor; UBF, upstream binding factor; $\dot{V}O_{2peak}$, peak volume of oxygen uptake; W_{peak}, peak aerobic power.

4. Introduction

Simultaneously incorporating both resistance (RT) and endurance training into a periodised training program, termed concurrent training (Leveritt *et al.*, 1999), can attenuate RT adaptations such as muscle hypertrophy, compared with RT performed alone (Hickson, 1980; Kraemer *et al.*, 1995; Bell *et al.*, 2000). This is potentially mediated by an altered balance between post-exercise skeletal muscle protein synthesis (MPS) and breakdown, subsequently attenuating lean mass accretion. The mechanistic target of rapamycin complex 1 (mTORC1) is a key mediator of load-induced increases in MPS and subsequently muscle hypertrophy (Bodine *et al.*, 2001b; Drummond *et al.*, 2009). The activity of mTORC1 is antagonised by activation of the 5' adenosine monophosphate-activated protein kinase (AMPK), which acts to restore perturbations in cellular energy balance by inhibiting anabolic cellular processes and stimulating catabolism (Kimball, 2006). For example, in rodent skeletal muscle, low-frequency electrical stimulation mimicking endurance exercise-like contractions promotes AMPK activation and inhibition of mTORC1 signalling (Atherton *et al.*, 2005).

Subsequent work in humans (Carrithers *et al.*, 2007; Coffey *et al.*, 2009a; Coffey *et al.*, 2009b; Donges *et al.*, 2012; Lundberg *et al.*, 2012; Apro *et al.*, 2013; Fernandez-Gonzalo *et al.*, 2013; Lundberg *et al.*, 2014; Apro *et al.*, 2015; Pugh *et al.*, 2015) has focused on the hypothesis that attenuated muscle hypertrophy with concurrent training (Kraemer *et al.*, 1995; Bell *et al.*, 2000; Wilson *et al.*, 2012) may be explained by AMPK-mediated inhibition of the mTORC1 pathway. Several studies, however, have demonstrated that single sessions of concurrent exercise do not compromise either mTORC1 signalling or rates of MPS (Carrithers *et al.*, 2007; Donges *et al.*, 2012; Apro *et al.*, 2013; Apro *et al.*, 2015; Pugh *et al.*, 2015), and may even potentiate these responses (Lundberg *et al.*, 2012), compared with resistance exercise (RE) performed alone. However, a limitation of these studies is that most have examined these responses in either untrained individuals (Carrithers *et al.*, 2007; Donges *et al.*, 2012; Pugh *et al.*, 2015) or those who are relatively unaccustomed to the exercise protocol (Lundberg *et al.*,

2012; Fyfe *et al.*, 2016b). Given short-term training increases the mode-specificity of post-exercise molecular responses (Wilkinson *et al.*, 2008; Vissing *et al.*, 2011), examining perturbations to molecular signalling and gene expression in relatively training-unaccustomed individuals may confound any insight into the potential molecular mechanisms responsible for interference following concurrent training (Fyfe *et al.*, 2014).

Transient changes in translational efficiency (i.e., rates of protein synthesis per ribosome) after single sessions of concurrent exercise, as indexed by skeletal muscle mTORC1 signalling or rates of MPS, in relatively training-unaccustomed individuals therefore do not appear to explain interference to muscle hypertrophy following longer-term concurrent training. However, rates of cellular protein synthesis are determined not only by transient changes in translational efficiency, but also by cellular translational capacity (i.e., amount of translational machinery per unit of tissue, including ribosomal content) (Chaillou *et al.*, 2014). Ribosomes are supramolecular ribonucleoprotein complexes functioning at the heart of the translational machinery to convert mRNA transcripts into protein (Chaillou *et al.*, 2014), and ribosomal content dictates the upper limit of cellular protein synthesis (Iadevaia *et al.*, 2014). Early rises in protein synthesis in response to anabolic stimuli (e.g., a single bout of RE) are generally thought to be mediated by transient activation of existing translational machinery, whereas prolonged anabolic stimuli (e.g., weeks to months of RE training) induces an increase in total translational capacity via ribosome biogenesis (Chaillou *et al.*, 2014).

Ribosome biogenesis is a complex, well-orchestrated process involving transcription of the polycistrionic 45S rRNA (ribosomal RNA) precursor (45S pre-rRNA), processing of the 45S pre-rRNA into several smaller rRNAs (18S, 5.8S and 28S rRNAs), assembly of these rRNAs and other ribosomal proteins into ribosomal subunits (40S and 60S), and nuclear export of these ribosomal subunits into the cytoplasm (Thomson *et al.*, 2013; Chaillou *et al.*, 2014). The synthesis of the key

components of the ribosomal subunits is achieved via the coordinated actions of three RNA polymerases (RNA Pol-I, -II, and -III). The RNA Pol-I is responsible for the transcription of the 45S pre-rRNA in the nucleolus, which is considered the rate-limiting step in ribosome biogenesis (Moss & Stefanovsky, 1995). The 45S pre-rRNA is subsequently cleaved into the 18S, 5.8S and 28S rRNAs, which undergo post-transcriptional modifications via interactions with small nuclear ribonucleoproteins and several protein processing factors. The RNA Pol-II is responsible for the transcription of ribosomal protein-encoding genes, whereas RNA Pol-III mediates the nucleoplasmic transcription of 5S rRNA and tRNAs (transfer RNAs) (Thomson *et al.*, 2013).

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

Studies in both human (Nader *et al.*, 2014; Figueiredo *et al.*, 2015; Stec *et al.*, 2015) and rodent skeletal muscle (Adams *et al.*, 2002; Goodman *et al.*, 2011a; Miyazaki *et al.*, 2011; Chaillou *et al.*, 2012; von Walden *et al.*, 2012; Chaillou *et al.*, 2013) suggest ribosome biogenesis, as indexed by increases in total RNA content (>85% of which comprises rRNA) (Chaillou *et al.*, 2014), and increased mRNA expression of several RNA Pol-I regulatory factors, including UBF, cyclin D1 and TIF-1A, occurs concomitantly with muscle hypertrophy. In addition, attenuated rodent skeletal muscle hypertrophy with ageing (Kirby *et al.*, 2015; Stec *et al.*, 2015) and rapamycin treatment (Goodman *et al.*, 2011a) is associated with reduced markers of ribosome biogenesis, suggesting translational capacity is closely linked to the regulation of skeletal muscle mass. Despite the links between skeletal muscle hypertrophy and ribosome biogenesis (Chaillou *et al.*, 2014; Nader *et al.*, 2014; Figueiredo *et al.*, 2015), studies investigating molecular interference following concurrent exercise in human skeletal muscle have only measured transient (<6 h) post-exercise changes in translational efficiency (as indexed by mTORC1 signalling) and MPS (Carrithers *et al.*, 2007; Coffey *et al.*, 2009a; Coffey *et al.*, 2009b; Donges *et al.*, 2012; Lundberg *et al.*, 2012; Apro *et al.*, 2013; Fernandez-Gonzalo *et al.*, 2013; Lundberg *et al.*, 2014; Apro *et al.*, 2015; Pugh *et al.*, 2015). No studies have investigated changes in ribosome biogenesis either after single bouts of concurrent exercise or following periods of concurrent training. Whether attenuated muscle hypertrophy following concurrent training could be explained, at least in part, by attenuated ribosome biogenesis is unknown.

The aim of this study was therefore to investigate changes in markers of ribosome biogenesis and mTORC1 signalling after eight weeks of concurrent training compared with RT undertaken alone. A secondary aim was to determine the potential role of endurance training intensity in modulating skeletal muscle ribosome biogenesis adaptation to concurrent training, by comparing concurrent training incorporating either high-intensity interval training (HIT) or work-matched moderate-intensity continuous training (MICT). The induction of these responses in skeletal muscle was also

189 investigated following a single exercise session performed post-training. It was hypothesised that
190 compared with RT alone, concurrent training would attenuate the training-induced increase in markers
191 of skeletal muscle ribosome biogenesis, but not mTORC1 signalling, both at rest post-training and
192 after a single training session performed in a training-accustomed state. It was further hypothesised
193 that concurrent training incorporating HIT would preferentially attenuate training-induced skeletal
194 muscle hypertrophy relative to RT alone, and this would be associated with an attenuation of markers
195 of skeletal muscle ribosome biogenesis.
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197 ****** INSERT FIGURE 1 ABOUT HERE ******
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5. Methodology

Ethical approval

All study procedures conformed to the *Declaration of Helsinki* and were approved by the Victoria University Human Research Ethics Committee (HRE 13-309). After being fully informed of study procedures and screening for possible exclusion criteria, participants provided written informed consent.

Experimental overview

Participant details and procedures performed in this study have been previously described (Fyfe *et al.*, 2016a); however, these are briefly summarised as follows. The study employed a repeated-measures, parallel-group design (Figure 2A). After preliminary testing for maximal (one-repetition maximum [1-RM]) strength, aerobic fitness ($\dot{V}O_{2peak}$, the lactate threshold [LT] and peak aerobic power [W_{peak}]), and body composition (dual-energy x-ray absorptiometry [DXA]), participants were ranked by baseline 1-RM leg press strength and randomly allocated to one of three training groups. Each group performed training sessions that consisted of either 1) high-intensity interval training (HIT) cycling combined with RT (HIT+RT group, n = 8), 2) moderate-intensity continuous training (MICT) cycling combined with RT (MICT+RT group, n = 7) or 3) RT performed alone (RT group, n = 8).

After preliminary testing, and immediately prior to the first training session (i.e., at least 72 h after completion of preliminary testing), a resting muscle biopsy (PRE-T) was obtained from the *vastus lateralis* using the percutaneous needle biopsy technique (Bergstrom, 1962) modified with suction (Evans *et al.*, 1982). Participants then completed 8 weeks of group-specific training performed three times per week. Between 48 and 72 h after completing the post-training 1-RM strength testing, participants underwent a final group-specific training session (Figure 2B) whereby early post-exercise molecular responses in skeletal muscle were measured in a training-accustomed state. Three additional

biopsies [at rest (POST-T), and 1 h (+1 h) and 3 h (+3 h) post-exercise] were obtained during the final group-specific training session.

****** INSERT FIGURE 2 ABOUT HERE ******

Training intervention

The training intervention in this study has previously been described in detail (Fyfe *et al.*, 2016a). Briefly, participants began the 8-week training intervention 3 to 5 days after completion of preliminary testing. All training groups performed an identical RT program on non-consecutive days (typically Monday, Wednesday, and Friday), with the HIT+RT and MICT+RT groups completing the corresponding form of endurance exercise 10 min prior to commencing each RT session.

Final training session

Two or three days after completion of the training intervention and post-testing, participants perfomed a final group-specific training session (Figure 2B) whereby early post-exercise skeletal muscle responses were measured in a training-accustomed state. Participants reported to the laboratory after an overnight (~8-10 h) fast. After resting quietly for ~15 min upon arrival at the laboratory, a venous cathether was inserted into an anticubital forearm vein and a resting blood sample was obtained. A resting, post-training (POST-T) muscle biopsy was then taken from the *vastus lateralis* muscle (described subsequently). Participants in the RT group waited quietly for 10 min after the POST-T biopsy and then completed a standardised RT protocol (8 x 5 leg press repetitions at 80% of the post-training 1RM, 3 min recovery between sets). Participants in the HIT+RT and MICT+RT groups preceded the standardised RT with either HIT (10 x 2-min intervals at 140% of the post-training LT, 1 min passive recovery between intervals) or work- and duration-matched MICT cycling (30 min at 93.3% post-training LT), respectively. Fifteen minutes of passive recovery was allowed between

completion of either HIT or MICT and the subsequent RT bout. Each cycling bout was performed after a standardised warm-up ride at 75 W for 5 min. After completion of RT, participants rested quietly in the laboratory and additional biopsies were obtained after 1 (+1 h) and 3 h (+3 h) of recovery. Venous blood samples were also obtained at regular intervals during cycling and following recovery from both cycling and RT (Figure 2B).

Muscle sampling

After administration of local anaesthesia (1% Xylocaine), a small incision (~7 mm in length) was made through the skin, subcutaneous tissue, and fascia overlying the *vastus lateralis* muscle for each subsequent biopsy. A 5-mm Bergström needle was then inserted into the muscle and a small portion of muscle tissue (~50-400 mg) removed. All biopsies were obtained from separate incision sites in a distal-to-proximal fashion on the same leg as the pre-training biopsy. Muscle samples were blotted on filter paper to remove excess blood, immediately frozen in liquid nitrogen, and stored at –80°C until subsequent analysis. A small portion of each biopsy sample (~20 mg) was embedded in Tissue-Tek (Sakura, Finetek, NL), frozen in liquid nitrogen-cooled isopentane, and stored at –80°C for subsequent immunofluorescence analysis.

Western blotting

Approximately 5 mg of frozen muscle tissue was homogenised in lysis buffer (0.125M Tris-HCl, 4% SDS, 10% Glycerol, 10mM EGTA, 0.1M DTT, 1% protease/phosphatase inhibitor cocktail), left for 1 h at room temperature, and then stored overnight at -80°C. The following morning, samples were thawed and the protein concentration determined (Red 660 Protein Assay Kit, G-Biosciences, St. Louis, MO). Bromophenol blue (0.1%) was then added to each sample, which were then stored at -80°C until subsequent analysis. Proteins (8 µg) were separated by SDS-PAGE using 6-12% acrylamide pre-cast gels (TGX Stain Free, Bio-Rad laboratories, Hercules, CA) in 1× running buffer (25 mM Tris,

192 mM Glycine, 0.1% SDS), and transferred to polyvinylidine fluoride (PVDF) membranes (Bio-Rad laboratories, Hercules, CA) using a semi-dry transfer system (Trans Blot Turbo, Bio-Rad laboratories, Hercules, CA) for 7 min at 25 V. After transfer, membranes were blocked with 5% skim milk in 1×TBST (200 mM Tris, 1.5 M NaCl, 0.05% Tween 20) for 1 h at room temperature, washed with 1×TBST (5×5 min), and incubated with primary antibody solution (5% BSA [bovine serum albumin], 0.05% Na Azide in 1×TBST) overnight at 4°C. Primary antibodies for phosphorylated (p-) p-mTOR^{Ser2448} (1:1000; #5536), mTOR (1:1000), p-p70S6K1^{Thr389} (1:1000; #9234), p70S6K1 (1:1000), p-4E-BP1$^{Thr37/46}$ (1:1000; #2855), 4E-BP1 (1:1000; #9452), p-AMPK^{Thr172} (1:1000; #2535), AMPK (1:1000; #2532), p-rps6$^{Ser235/236}$ (1:750; #4856), rps6 (1:1000; #2217) and p-ACC^{Ser79} (1:1000; #3661) were from Cell Signalling Technology (Danvers, MA), p-UBF^{Ser388} (1:1000; sc-21637-R), UBF (1:000; sc-9131) and cyclin D1 (1:1000; sc-450) were from Santa Cruz Biotechnology (Dallas, TX), and p-RRN3 (TIF-1A)^{Ser649} (1:1000; ab138651) and TIF-1A (1:1000; ab70560) were from Abcam (Cambridge, UK). The following morning, membranes were washed again with 1×TBST and incubated with a secondary antibody (Perkin Elmer, Waltham, MA, #NEF812001EA; 1:50000 or 1:100000 in 5% skim milk and 1×TBST) for 1 h at room temperature. After washing again with 1×TBST, proteins were detected with chemiluminescence (SuperSignal^{TM} West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific, Waltham, MA) and quantified via densitometry (Image Lab 5.0, Bio-Rad laboratories, Hercules, CA). All sample timepoints for each participant were run on the same gel and normalised to both an internal pooled sample present on each gel, and the total protein content of each lane using a stain-free imaging system (Chemi Doc^{TM} MP, Bio-Rad laboratories, Hercules, CA). Phosphorylated proteins were then expressed relative to the total amount of each respective protein.

Real-time quantitative PCR (qPCR)

RNA extraction

Total RNA (1145 ± 740 ng; mean ± SD) was extracted from approximately 25 mg of muscle tissue using TRI Reagent® (Sigma Aldrich, St. Louis, MO) according to the manufacturer's protocol. Muscle samples were firstly homogenised in 500 µL of TRI Reagent® using a Tissue Lyser II and 5 mm stainless steel beads (Qiagen, Venlo, Limburg, Netherlands) for 120 s at 30 Hz. After resting for 5 min on ice, 50 µL of 1-bromo-3-chloropropane (BCP) was added to the tube, inverted for 30 s to mix, and then rested for 10 min at room temperature. The homogenate was then centrifuged for 15 min at 13,000 rpm and the upper transparent phase transferred to another tube. Isopropanol (400 µL) was added to the tube, inverted briefly to mix, and stored overnight at -20°C to precipitate the RNA. After overnight incubation, the solution was centrifuged for 60 min at 13,000 rpm and at 4°C to pellet the RNA. The RNA pellet was washed twice by centrifugation in 75% ethanol/nuclease-free water (NFW) for 15 min at 13,000 rpm, allowed to air-dry, and then dissolved in 15 µL of NFW (Ambion Inc., Austin, TX). The quantity and quality of RNA was subsequently determined using a spectrophotometer (NanoDrop One, Thermo Scientific, Wilmington, DE). The purity of RNA was assessed using the ratio between the absorbance at 260 nm and absorbance at 280 nm (mean ± SD; 2.37 ± 0.43), and the ratio between the absorbance at 260 nm and absorbance at 230 nm (1.71 ± 0.42). The total skeletal muscle RNA concentration was calculated based on the total RNA yield relative to the wet weight of the muscle sample.

Reverse transcription

For mRNA analysis, first-strand cDNA was generated from 1 µg RNA in 20 µL reaction buffer using the iScript® cDNA synthesis kit (Bio-Rad laboratories, Hercules, CA) according to manufacturer's protocol, with each reaction comprising 4 µL 5× iScript reaction mix, 1 µL iScript Reverse Transcriptase, 5 µL NFW and 10 µL of RNA sample (100 ng/µL). Reverse transcription was then

performed with the following conditions: 5 min at 25°C to anneal primers, 30 min at 42°C for the extension phase, and 5 min at 85°C. Following reverse transcription, samples were DNase-treated (Life Technologies, Carlsbad, CA) and cDNA was stored at -20°C until further analysis.

Real-time quantitative PCR (qPCR)

Real-time PCR was performed using a Realplex^2 PCR system (Eppendorf, Hamburg, Germany) to measure mRNA levels of MuRF-1 (muscle RING-finger 1), Atrogin-1 (muscle atrophy f-box), FoxO1 (forkhead box-O1), PGC-1α (peroxisome proliferator-activated gamma receptor co-activator-1 alpha), UBF, TIF-1A, cyclin D1, POLR1B, and commonly used reference genes GAPDH (glyceraldehyde 3-phosphate dehydrogenase), cyclophilin (also known as peptidyl-prolylcis-trans isomerase), β2M (beta-2 microglobulin) and TBP (TATA binding protein). Target rRNAs were the mature ribosome species 5.8S, 18S and 28S. Since primers specific for these mature rRNA sequences will also amplify pre-RNA transcripts (i.e., the 45S pre-rRNA), we used specifically designed primers (QIAGEN, Venlo, Limburg, The Netherlands) to distinguish between mature rRNA species and those still bound to the 45S pre-rRNA transcript, as previously described (Figueiredo *et al.*, 2015). Briefly, primers were designed specifically for pre-rRNA sequences spanning the 5'end external/internal transcribed spacer regions (ETS and ITS, respectively) of the 45S pre-RNA transcript and the internal regions of mature rRNA sequences (i.e., 18S-ETS, 5.8S-ITS, and 28S-ETS). For clarity, primers amplifying the mature rRNA transcripts are henceforth designated as 'mature' transcripts (e.g., 18S rRNA [mature]), as opposed to those primers amplifying rRNA sequences bound to the 45S rRNA precursor, henceforth designated as 'span' transcripts (e.g., 18S rRNA [span]). A specific primer for the initial region of the 5' end of the 45S pre-rRNA transcript was used to measure 45S pre-rRNA expression levels (Figueiredo *et al.*, 2015). Standard and melting curves were performed for all primers to ensure both single-product and amplification efficiency. Details for all primers used are provided in Table 1 (mRNA) and Table 2 (rRNA).

****** INSERT TABLE 1 ABOUT HERE ******

****** INSERT TABLE 2 ABOUT HERE ******

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

Immunohistochemistry

Muscle cross-sections (10 µM) were cut at -20°C using a cryostat (Microm HM 550, Thermo Fisher Scientific, Waltham, MA), mounted on uncoated glass slides, and air-dried for 20 min at room temperature. Sections were then rinsed briefly with 1×PBS (0.1M; Sigma Aldrich, St Louis, MO), fixed with cold paraformaldehyde (4% in 1×PBS) for 10 min at room temperature, rinsed three times with 1×PBS, incubated in 0.5% TritonX in 1×PBS for 5 min at room temperature, rinsed again three times with 1×PBS, and then blocked for 1 h at room temperature in a 3% BSA solution in 1×PBS. After blocking, sections were then incubated with a primary antibody for myosin heavy chain type I

(A4.840, Developmental Studies Hybridoma Bank, University of Iowa, IA), diluted 1:25 in 3% BSA/PBS overnight at 4°C. The following morning, sections were washed four times in 1×PBS for 10 min each, before incubating with a secondary antibody (Alexa Fluor® 488 conjugate Goat anti-mouse IgM, cat. no. A-21042, Thermo Fisher Scientific, Waltham, MA) diluted 1:200 in 3% BSA/PBS for 2 h at room temperature. Sections were again washed four times in 1×PBS for 10 min each, before incubation with Wheat Germ Agglutinin (WGA) (Alexa Fluor® 594 Conjugate; cat. no. W11262, Thermo Fisher Scientific, Waltham, MA), diluted to 1:100 in 1×PBS (from a 1.25 mg/mL stock solution), for 15 min at room temperature. Sections were washed again 4 times with 1×PBS for 3 min each, blotted dry with a Kim-Wipe, and Flouroshield^{TM} (cat. no. F6182; Sigma Aldrich, St Louis, MO) added to each section before the coverslip was mounted. Stained muscle sections were air-dried for ~2 h and viewed with an Olympus BX51 microscope coupled with an Olympus DP72 camera for flourescence detection (Olympus, Shinjuku, Japan). Images were captured with a 10× objective and analysed using Image Pro Premier software (version 9.1; Media Cybernetics, Rockville, MD). Analysis was completed by an investigator blinded to all groups and time points. For each subject, muscle fibre CSA was determined for both type I and type II muscle fibres. For the RT, HIT+RT and MICT+RT groups, a total of 107 ± 61, 112 ± 67, and 84 ± 73 (mean ± SD) type I fibres and 154 ± 72, 136 ± 80, and 144 ± 76 (mean ± SD) type II fibres were included for analysis, respectively.

6. Statistical analyses

The effect of training group on outcomes was evaluated via a two-way (time × group) analysis of variance with repeated-measures (RM-ANOVA) (SPSS, Version 21, IBM Corporation, New York, NY). Western blot, qPCR and immunohistochemistry data were log-transformed before analysis to reduce non-uniformity of error (Hopkins *et al.*, 2009). To quantify the magnitude of within- and between-group differences for dependent variables, a magnitude-based approach to inferences using the standardised difference (effect size, ES) was used (Hopkins *et al.*, 2009). The magnitude of effects were defined according to thresholds suggested by Hopkins (Hopkins *et al.*, 2009), whereby <0.2 = trivial, 0.2-0.6 = small, 0.6-1.2 = moderate, 1.2-2.0 = large, 2.0-4.0 = very large and >4.0 = extremely large effects. Lacking information on the smallest meaningful effect for changes in protein phosphorylation and gene expression, the threshold for the smallest worthwhile effect was defined as an ES of 0.4, rather than the conventional threshold of 0.2 (Fyfe *et al.*, 2016b). Magnitude-based inferences about effects were made by qualifying the effects with probabilities reflecting the uncertainty in the magnitude of the true effect (Batterham & Hopkins, 2005). Effects that were deemed substantial in magnitude (and therefore meaningful) were those at least 75% '*likely*' to exceed the smallest worthwhile effect (according to the overlap between the uncertainty in the magnitude of the true effect and the smallest worthwhile change (Batterham & Hopkins, 2005)). Exact *P* values were also determined for each comparison, derived from paired (for within-group comparisons) or unpaired (for between-group comparisons) *t*-tests, with a Bonferroni correction applied to correct for multiple comparisons (SPSS, Version 21, IBM Corporation, New York, NY). A summary of all within- and between-group comparisons for this study are presented in supplementary tables 1 and 2, respectively. Physiological (blood lactate, blood glucose, heart rate) and psychological (rating of perceived exertion [RPE]) responses to exercise are reported as mean values ± SD, whereas protein phosphorylation and gene expression data are reported as mean within- and between-condition percentage differences ±90 % CL.

7. Results

Training-induced changes in maximal strength and lean mass

In brief, and as previously reported (Fyfe *et al.*, 2016a), 1-RM leg press strength was improved from PRE-T to POST-T for RT (mean change ±90% confidence interval; 38.5 ±8.5%; effect size [ES] ±90% confidence interval; 1.26 ±0.24; $P<0.001$), HIT+RT (28.7 ±5.3%; ES, 1.17 ±0.19; $P<0.001$) and MICT+RT (27.5 ±4.6%, ES, 0.81 ±0.12; $P<0.001$); however, the magnitude of this change was greater for RT vs. both HIT+RT (7.4 ±8.7%; ES, 0.40 ±0.40) and MICT+RT (8.2 ±9.9%; ES, 0.60 ±0.45). There were no substantial between-group differences in 1-RM bench press strength gain. Lower-body lean mass was similarly increased for RT (4.1 ±2.0%; ES; 0.33 ±0.16; $P=0.023$) and MICT+RT (3.6 ±2.4%; ES; 0.45 ±0.30; $P=0.052$); however, this increase was attenuated for HIT+RT (1.8 ±1.6%; ES; 0.13 ±0.12; $P=0.069$).

Physiological and psychological responses to the final training session

Heart rate and rating of perceived exertion (RPE)

During the final training session, there was a higher average heart rate (mean difference range, 14 ±12 to 19 ±14 bpm; ES, 1.04 ±0.88 to 1.22 ±0.89; $P \leq 0.043$; Table 3) and rating of perceived exertion (RPE) (2 ±2 to 4 ±2 AU; ES, 1.51 ±0.86 to 2.15 ±0.87; $P \leq 0.06$) for HIT compared with MICT.

Venous blood lactate and glucose responses during the final training session

During the final training session, venous blood lactate (Table 3) was higher for HIT compared with MICT at all time points both during cycling (mean difference range, 0.8 ±0.5 to 4.5 ±1.1 mmol·L^{-1}; ES range, 1.46 ±0.87 to 3.65 ±0.85; $P \leq 0.01$) and during the 15-min recovery period after cycling (3.5 ±1.0 to 5.0 ±1.2 mmol·L^{-1}; ES, 3.11 ±0.85 to 3.68 ±0.85; $P < 0.001$). Venous blood glucose (Table 3) was also higher for HIT compared with MICT after 16, 22, 28 and 34 min cycling (0.4 ±0.7 to 1.6 ±0.9

446 mmol·L^{-1}; ES, 0.54 ±0.86 to 1.52 ±0.86; $P \leq 0.039$), and during the 15-min recovery period after
447 cycling (0.9 ±0.7 to 1.8 ±1.0 mmol·L^{-1}; ES, 1.11 ±0.85 to 1.50 ±0.85; $P \leq 0.041$).
448
449 After completion of RE performed as part of the final training session, venous blood lactate (Table 4)
450 was higher for HIT+RT compared with RT after 0, 2, 5, 10, 60, 90 and 180 min of recovery (0.1 ±0.1
451 to 1.4 ±0.9 mmol·L^{-1}; ES, 0.80 ±0.84 to 1.74 ±0.84; $P \leq 0.095$), and higher for HIT+RT compared
452 with MICT+RT at all timepoints (0.1 ±0.1 to 1.1 ±1.4 mmol·L^{-1}; ES, 0.73 ±0.87 to 1.82 ±0.86; $P \leq$
453 0.161). Post-RE venous blood glucose (Table 4) was lower for HIT+RT compared with RT after 2, 10,
454 and 30 min of recovery (0.3 ±0.2 to 0.3 ±0.3 mmol·L^{-1}; ES, -0.65 ±0.84 to -1.02 ±0.84; $P \leq 0.193$),
455 and higher for HIT+RT compared with RT after 60 min of recovery (0.4 ±0.4 mmol·L^{-1}; ES, 0.88
456 ±0.84; $P = 0.077$). Blood glucose was higher for MICT compared with HIT+RT at +30 min of recovery
457 (0.3 ±0.2 mmol·L^{-1}; ES, 1.29 ±0.86; $P = 0.021$), and lower for HIT+RT compared with MICT+RT at
458 +60 min of recovery (0.2 ±0.2 mmol·L^{-1}; ES, -1.09 ±0.85; $P = 0.045$).
459
460 ****** INSERT TABLE 3 ABOUT HERE******
461
462 ****** INSERT TABLE 4 ABOUT HERE******

463 **Protein signalling responses**

464 *Ribosome biogenesis signalling*

465 **p-TIF-1A^{Ser649}**. There was a main effect of time for TIF-1A^{Ser649} phosphorylation ($P < 0.001$).
466 At POST-T, TIF-1A phosphorylation was higher compared with PRE-T for HIT+RT (133
467 ±102%; ES, 0.62 ±0.31; $P = 0.047$; Figure 3A), but unchanged for RT or MICT+RT. Compared
468 with POST-T, TIF-1A phosphorylation was higher for RT at +1 h (123 ±79%; ES, 0.45 ±0.19;
469 $P = 0.002$), and +3 h (241 ±315%; ES, 0.69 ±0.46; $P = 0.017$), but unchanged for HIT+RT or
470 MICT+RT. The change in TIF-1A phosphorylation between POST-T and +3 h was greater for
471 RT compared with both HIT+RT (52 ±46%; ES, 0.76 ±0.89) and MICT+RT (75 ±24%; ES,
472 1.31 ±0.80), and lower for MICT+RT vs. HIT+RT (-47 ±36%; ES, -0.69 ±0.70).

473

474 **p-UBF^{Ser388}**. There were main effects of time ($P < 0.001$), group ($P = 0.004$), and a time ×
475 group interaction ($P < 0.001$), for changes in UBF^{Ser388} phosphorylation. The phosphorylation
476 of UBF^{Ser388} was unchanged at POST-T compared with PRE for all training groups (see Figure
477 3B). Compared with POST-T, UBF phosphorylation was increased for RT at both +1 h (78
478 ±58%; ES, 0.82 ±0.45; $P = 0.010$) and + 3 h (125 ±72%; ES, 1.15 ±0.45; $P = 0.001$), but
479 unchanged for either HIT+RT or MICT+RT. The change in UBF phosphorylation between
480 POST-T and +1 h was greater for RT compared with both HIT+RT (32 ±23%; ES, 0.54 ±0.46)
481 and MICT+RT (37 ±27%; ES, 0.61 ±0.55), and greater between POST-T and +3 h for RT
482 compared with both HIT+RT (49 ±17%; ES, 0.92 ±0.45) and MICT+RT (64 ±12%; ES, 1.35
483 ±0.42).

484

485 **Cyclin D1 protein.** There were main effects of time ($P < 0.001$) and group ($P = 0.008$) for
486 changes in cyclin D1 protein content. Protein content of cyclin D1 was unchanged between

PRE-T and POST-T for all training groups (Figure 3C). For HIT+RT, cyclin D1 protein content was reduced at +1 h compared with POST-T (-34 ±7%; ES, -0.66 ±0.16; P = 0.008).

****** INSERT FIGURE 3 ABOUT HERE******

AMPK/mTORC1 signalling

p-AMPK^{Thr172}. There was a main effect of time for AMPK^{Thr172} phosphorylation (P = 0.033). The phosphorylation of AMPK^{Thr172} was unchanged at POST-T compared with PRE-T for all training groups (Figure 4A). AMPK phosphorylation was, however, increased at +1 h compared with POST-T for RT (78 ±72%; ES, 0.34 ±0.23; P = 0.031). The change in AMPK phosphorylation between POST-T and +3 h was also greater for RT compared with MICT+RT (59 ±44%; ES, 0.79 ±0.83) but not HIT+RT (54 ±49%; ES, 0.69 ±0.83).

p-ACC^{Ser79}. There was a time × group interaction for ACC^{Ser79} phosphorylation (P = 0.04). The phosphorylation of ACC^{Ser79} was unchanged at POST-T compared with PRE-T for all training groups (Figure 4B). Compared with POST-T, ACC phosphorylation was reduced at +1 h for both RT (-36 ±22%; ES, -0.28 ±0.20; P = 0.026) and MICT+RT (46 ±20%; ES, -0.56 ±0.33; P = 0.016), and reduced at +3 h compared with POST-T for RT (45 ±20%; ES, -0.37 ±0.22; P = 0.012). Compared with RT, the change in ACC phosphorylation was also greater for HIT+RT between POST-T and both +1 h (99 ±100%; ES, 0.65 ±0.46) and +3 h (169 ±168%; ES, 0.94 ±0.56).

p-mTOR^{Ser2448}. There was a main effect of time for mTOR ^{Ser2448} phosphorylation (P = 0.001). The phosphorylation of mTOR^{Ser2448} was unchanged at POST-T compared with PRE-T for all training groups (Figure 4C). Compared with POST-T, mTOR phosphorylation was increased

512 at +1 h for RT (105 ±137%; ES, 0.46 ±0.40; *P* = 0.048), but not for either HIT+RT (30 ±71%;
513 ES, 0.32 ±0.62; *P* = 0.320) or MICT+RT (77 ±184%; ES, 0.37 ±0.59; *P* = 0.218), and increased
514 at +3 h for compared with POST-T for HIT+RT (70 ±45%; ES, 0.64 ±0.31; *P* = 0.030). There
515 were no substantial between-group differences in mTOR phosphorylation at any time point.
516
517 **p-p70S6K1^{Thr389}.** There was a main effect of time for p70S6K1^{Thr389} phosphorylation (*P* <
518 0.001). The phosphorylation of p70S6K1^{Thr389} was increased at POST-T compared with PRE
519 for HIT+RT (95 ±47%; ES, 0.66 ±0.24; *P* = 0.024; Figure 4D), but not for RT or MICT+RT.
520 Compared with POST-T, p70S6K1 phosphorylation was increased by RT at +1 h (78 ±77%;
521 ES, 0.51 ±0.37; *P* = 0.026) but was unchanged for HIT+RT or MICT+RT. The change in
522 p70S6K1 phosphorylation between POST-T and +3 h was also substantially greater for RT
523 compared with both HIT+RT (47 ±50%; ES, 0.86 ±1.13) and MICT+RT (50 ±46%; ES, 0.88
524 ±1.05).
525
526 **p-rps6$^{Ser235/236}$.** There was a main effect of time for rps6$^{Ser235/236}$ phosphorylation (*P* < 0.001).
527 The phosphorylation of rps6$^{Ser235/236}$ was unchanged at POST-T compared with PRE-T for all
528 training groups (Figure 4E). Compared with POST-T, rps6 phosphorylation was increased for
529 all training groups at +1 h (RT: 700 ±678%; ES, 0.75 ±0.28; *P* < 0.001; HIT+RT: 475 ±572%;
530 ES, 0.66 ±0.33; *P* = 0.005; MICT+RT: 621 ±420%; ES, 1.49 ±0.42; *P* < 0.001) and +3 h (RT:
531 967 ±1047%; ES, 0.85 ±0.31; *P* < 0.001; HIT+RT: 294 ±319%; ES, 0.51 ±0.28; *P* = 0.006;
532 MICT+RT: 176 ±200%; ES, 0.76 ±0.51; *P* = 0.026). The change in rps6 phosphorylation
533 between POST-T and +3 h was, however, substantially greater for RT compared with
534 MICT+RT (74 ±29%; ES, 0.72 ±0.51) but not HIT+RT (63 ±41%; ES, 0.57 ±0.56).
535

p-4E-BP1$^{Thr56/47}$. There was a main effect of group for 4E-BP1$^{Thr36/47}$ phosphorylation (P < 0.001; Figure 4F); however, there were no between-group differences in 4E-BP1$^{Thr36/47}$ phosphorylation at any time point.

****** INSERT FIGURE 4 ABOUT HERE ******

****** INSERT FIGURE 5 ABOUT HERE ******

Ribosomal RNA (rRNA) responses

Total RNA content. Total RNA content was used as an index of total translational capacity of skeletal muscle, since ribosomal RNA comprises over 85% of the total RNA pool (Haddad *et al.*, 2005). There was a time × group interaction for changes in total RNA content (P = 0.008). At PRE, total RNA content was higher for RT compared with both HIT+RT (38 ±17%; ES, -1.48 ±0.84; P = 0.005; Table 5) and MICT+RT (25 ±12%; ES, 1.47 ±0.85; P = 0.010). Total RNA content decreased between PRE-T and POST-T for RT (-11 ±5%; ES, -0.17 ±0.09; P = 0.025). Conversely, total RNA content was not substantally changed between PRE-T and POST-T for both HIT+RT (32 ±18%; ES, 0.30 ±0.15; P = 0.077) and MICT+RT (20 ±15%; ES, 0.12 ±0.08; P = 0.083). The PRE-T to POST-T change in total RNA content was, however, greater for both HIT+RT (48 ±39%; ES, 1.14 ±0.76) and MICT+RT (34 ±24%; ES, 1.24 ±0.75) compared with RT.

****** INSERT TABLE 5 ABOUT HERE ******

45S pre-rRNA. There was a main effect of time for changes in 45S pre-rRNA expression (P < 0.001). Expression of 45S pre-rRNA was unchanged at POST-T compared with PRE-T for all training groups (Figure 6); however, the change in 45S pre-rRNA expression between PRE-

562 T and POST-T was greater for both HIT+RT (58 ±76%; ES, 0.71 ±0.71) and MICT+RT (75
563 ±81%; ES, 0.85 ±0.68) compared with RT. There were no substantial changes nor between-
564 group differences in 45S pre-rRNA expression between POST-T and +3 h for either training
565 group.
566
567 ****** INSERT FIGURE 6 ABOUT HERE******
568
569 **5.8S rRNA (mature).** There was a main effect of time for changes in 5.85S rRNA expression
570 (P = 0.004). Expression of 5.85S rRNA was reduced at POST-T compared with PRE-T for RT
571 (-51 ±16%; ES, -0.69 ±0.31; P = 0.017; Figure 7A). The change in 5.8S rRNA expression
572 between PRE-T and POST-T was also greater for both HIT+RT (125 ±109%; ES, 1.27 ±0.73)
573 and MICT+RT (120 ±111%; ES, 0.99 ±0.61) compared with RT. There were no substantial
574 changes in 5.8S rRNA expression between POST-T and +3 h for either training group.
575
576 **5.8S rRNA (span).** There was a time × group interaction for changes in 5.85S (span) rRNA
577 expression (P = 0.008). Expression of 5.8S rRNA (span) was reduced at POST-T compared
578 with PRE-T for RT (-36 ±15%; ES, -0.51 ±0.27; P = 0.027; Figure 7B). The change in 5.8S
579 rRNA (span) expression between PRE-T and POST-T was also greater for HIT+RT compared
580 with RT (112 ±116%; ES, 1.40 ±0.97).
581
582 **18S rRNA (mature).** There was a main effect of group for changes in 5.85S rRNA expression
583 (P = 0.049). Expression of 18S rRNA was, however, not substantially different at any time
584 point, nor were there any substantial between-group differences in changes in 18S rRNA
585 expression (Figure 7C).
586

18S rRNA (span). There were no substantial effects of training or any between-group differences in changes in 18S rRNA (span) expression (Figure 7D), although a small increase in 18S rRNA (span) expression was noted at +3 h compared with POST-T for MICT+RT (63 ±48%; ES, 0.21 ±0.12; $P = 0.029$).

28S rRNA (mature). Expression of 28S rRNA was reduced at POST-T compared with PRE-T for RT (-33 ±15%; ES, -0.49 ±0.28; $P = 0.037$; Figure 7E); however, this effect was only possibly substantial. The change in 28S rRNA expression between PRE-T and POST-T was also greater for both HIT+RT (73 ±56%; ES, 1.23 ±0.71; $P = 0.007$) and MICT+RT (63 ±55%; ES, 1.10 ±0.74; $P = 0.023$) compared with RT. There were no substantial changes in 28S rRNA expression between POST-T and +3 h for either training group.

28S rRNA (span). There was a main effect of group for changes in 28S rRNA (span) expression ($P < 0.001$). There were no substantial changes in 28S rRNA (span) expression between PRE-T and POST-T for either training group (Figure 7F). However, the change in 28S rRNA (span) expression between PRE-T and POST-T was greater for HIT+RT compared with RT (123 ±109%; ES, 0.81 ±0.48).

****** INSERT FIGURE 7 ABOUT HERE ******

mRNA responses

TIF-1A mRNA. There was a main effect of time for changes in TIF-1A mRNA expression ($P = 0.008$). Expression of TIF-1A mRNA was unchanged at POST-T compared with PRE-T for all training groups (Figure 8A). Compared with POST-T, TIF-1A expression was increased at

612 +3 h for both RT (26 ±12%; ES, 0.53 ±0.21; *P* = 0.003) and MICT+RT (36 ±35%; ES, 0.59
613 ±0.50; *P* = 0.038), but not HIT+RT. There were no substantial between-group differences in
614 changes in TIF-1A expression.
615
616 **UBF mRNA.** There were main effects of time (*P* = 0.008) and group (*P* = 0.039) for changes
617 in UBF mRNA expression. Expression of UBF mRNA was unchanged at POST-T compared
618 with PRE-T for all training groups (Figure 8B). There were no substantial changes in UBF
619 expression between POST-T and +3 h for either training group.
620
621 **POLR1B mRNA.** There were main effects of time (*P* = 0.001) and a time × group interaction
622 (*P* = 0.007) for changes in POLR1B mRNA expression. Expression of POLR1B mRNA was
623 reduced at POST-T compared with PRE-T for RT (-26 ±16%; ES, -0.44 ±0.32; *P* = 0.026;
624 Figure 8C). Compared with POST-T, POLR1B expression was increased at +3 h for both
625 HIT+RT (44 ±42%; ES, 0.57 ±0.44; *P* = 0.047) and MICT+RT (48 ±43%; ES, 0.51 ±0.37; *P*
626 = 0.033), but unchanged for RT. The change in POLR1B mRNA expression between both
627 PRE-T -POST-T (37 ±30%; ES, 0.87 ±0.60) and POST-T -+3 h (34 ±51%; ES, 0.81 ±1.03)
628 was greater for HIT+RT vs. RT.
629
630 **Cyclin D1 mRNA.** There was a main effect of time for changes in cyclin D1 mRNA expression
631 (*P* = 0.007). Expression of cyclin D1 mRNA was increased for HIT+RT at POST-T compared
632 with PRE-T (101 ±54%; ES, 0.59 ±0.22; *P* = 0.001; Figure 8D). There were no substantial
633 changes in cyclin D1 mRNA expression between POST-T and +3 h for either training group.
634
635 ****** INSERT FIGURE 8 ABOUT HERE ******
636

637 **MuRF-1 mRNA.** There were main effects of time (P = 0.004) and a time × group interaction
638 (P = 0.019) for changes in MuRF-1 mRNA expression. Expression of MuRF-1 mRNA was
639 unchanged at POST-T compared with PRE for all training groups (Figure 9A). Compared with
640 POST-T, MuRF-1 expression was increased at +3 h for HIT+RT (206 ±163%; ES, 1.35 ±0.61;
641 P = 0.003), but unchanged for either MICT+RT and RT. The change in MuRF-1 expression
642 between POST-T and +3 h was greater for HIT+RT compared with both RT (168 ±176%; ES,
643 2.15 ±1.34) and MICT+RT (60 ±34%; ES, 1.85 ±1.56).
644
645 **Atrogin-1 mRNA.** There were main effects of time (P = 0.028) and a time × group interaction
646 (P = 0.049) for changes in Atrogin-1 mRNA expression. Atrogin-1 mRNA content was
647 unchanged at POST-T compared with PRE for all training groups (Figure 9B). Compared with
648 POST-T, Atrogin-1 expression was reduced at +3 h for RT (-44 ±22%; ES, -0.91 ±0.60; P =
649 0.018), but not substantially changed for either HIT+RT ot MICT+RT. The reduction in
650 Atrogin-1 mRNA expression between POST-T and +3 h was greater for RT compared with
651 both HIT+RT (-89 ±83%; ES, -1.22 ±0.82) and MICT+RT (-86 ±89%; ES, -1.14 ±0.85).
652
653 **Fox-O1 mRNA.** There was a main effect of time for changes in Fox-O1 mRNA expression (P
654 = 0.004). The mRNA levels of Fox-O1 was between PRE-T and POST-T for RT (28 ±17%;
655 ES, 0.49 ±0.27; P = 0.051), but unchanaged for HIT+RT and MICT+RT (Figure 9C). At +3 h,
656 Fox-O1 mRNA was increased compared with POST-T only for HIT+RT (158 ±65%; ES, 0.59
657 ±0.16; P < 0.001). The change in Fox-O1 mRNA expression between POST-T and +3 h was
658 also substantially greater for HIT+RT compared with both RT (141 ±73%; ES, 0.80 ±0.27) and
659 MICT+RT (47 ±31%; ES, 0.54 ±0.47).
660

PGC-1α mRNA. There were main effects of time ($P < 0.001$), group ($P < 0.001$), and a time × group interaction ($P < 0.001$), for changes in PGC-1α mRNA expression (Figure 9D). Compared with POST-T, PGC-1α mRNA expression was increased at +3 h for both HIT+RT (826 ±349%; ES, 4.58 ±0.76; $P < 0.001$) and MICT+RT (590 ±481%; ES, 1.97 ±0.66; $P = 0.001$), but unchanged for RT. The change in PGC-1α mRNA expression between POST-T and +3 h was greater for both HIT+RT (635 ±360%; ES, 4.80 ±1.14) and MICT+RT (447 ±379%; ES, 2.75 ±1.05) compared with RT.

****** INSERT FIGURE 9 ABOUT HERE ******

Muscle fibre CSA responses

Type I muscle fibre CSA (see Table 5) was increased at POST-T compared with PRE-T for RT (15 ±13%; ES, 0.10 ±0.08; $P = 0.035$), but was not substantially changed for either HIT+RT (-23 ±19%; ES, -0.09 ±0.08; $P = 0.135$) or MICT+RT (0.4 ±17%; ES, 0.00 ±-0.14; $P = 0.989$). The training-induced change in type I fibre CSA was also substantially greater for RT compared with HIT+RT (34 ±22%; ES, 1.03 ±0.80), but not MICT+RT (15 ±54%; ES, 0.39 ±1.45).

Type II muscle fibre CSA (see Table 5) was not substantially changed between PRE-T and POST-T for either RT (19 ±27%; ES, 0.09 ±0.12; $P = 0.139$), HIT+RT (0.4 ±24%; ES, 0.00 ±0.08; $P = 0.974$) or MICT+RT (16 ±14%; ES, 0.19 ±0.16; $P = 0.344$). There were no substantial differences in the training-induced changes in type II fibre CSA. Representative immunohistochemical images are shown in Figure 10.

****** INSERT FIGURE 10 ABOUT HERE ******

686 **8. Discussion**

687 Previous investigations on molecular responses and adaptations in skeletal muscle to
688 concurrent training have focused almost exclusively on markers of enhanced post-exercise
689 translational efficiency (i.e., mTORC1 signalling and rates of MPS) (Carrithers *et al.*, 2007;
690 Coffey *et al.*, 2009a; Coffey *et al.*, 2009b; Donges *et al.*, 2012; Lundberg *et al.*, 2012; Apro *et*
691 *al.*, 2013; Fernandez-Gonzalo *et al.*, 2013; Lundberg *et al.*, 2014; Apro *et al.*, 2015; Pugh *et*
692 *al.*, 2015). For the first time, we present data on the regulation of translational capacity (i.e.,
693 ribosome biogenesis) with concurrent training relative to RT performed alone, including
694 regulators of RNA Pol-I-mediated rDNA transcription and changes in expression levels of the
695 45S rRNA precursor and mature rRNA species (i.e., 5.8S, 18S, and 28S). The major findings
696 were that although a single bout of RE, when performed in a training-accustomed state, further
697 increased mTORC1 signalling and the phosphorylation of RNA Pol-I regulatory factors (TIF-
698 1A and UBF) compared with concurrent training, this was not associated with increased
699 expression of either the 45S rRNA precursor or mature rRNA species. Rather, changes in total
700 RNA content and expression of mature rRNAs (i.e., 5.8S, 28S) tended to be greater following
701 concurrent exercise, regardless of the endurance training intensity employed. These
702 observations contrast with our findings regarding training-induced changes in muscle fibre-
703 type specific hypertrophy, which was greater in type I muscle fibres for the RT group,
704 suggesting a disconnect between training-induced changes in markers of ribosome biogenesis
705 and skeletal muscle hypertrophy.
706
707 We employed a post-training exercise trial to investigate potential interference to mTORC1
708 signalling following exercise protocols that participants were accustomed to via eight weeks of
709 prior training. This was to overcome the limitation that most studies examining molecular
710 responses in skeletal muscle following a single concurrent exercise session have utilised

711 untrained or relatively training-unaccustomed participants (Carrithers *et al.*, 2007; Donges *et*
712 *al.*, 2012; Lundberg *et al.*, 2012; Pugh *et al.*, 2015). In contrast to previous investigations
713 (Carrithers *et al.*, 2007; Donges *et al.*, 2012; Lundberg *et al.*, 2012; Pugh *et al.*, 2015), we
714 observed enhanced mTORC1 signalling after RT compared with concurrent exercise, including
715 increased mTOR and p70S6K1 phosphorylation at 1 h post-exercise, and elevated rps6
716 phosphorylation at +3 h. These observations differ from previous data, including our own (Fyfe
717 *et al.*, 2016b), showing no differences in mTORC1 signalling between single bouts of either
718 RE, either performed alone or following a bout of continuous endurance exercise (Fernandez-
719 Gonzalo *et al.*, 2013). It has further been suggested that any small tendency for mTORC1
720 signalling responses (e.g., p70S6K^{Thr389} phosphorylation) to be enhanced by concurrent
721 exercise (relative to RE alone) before training, as shown in a previous study (Lundberg *et al.*,
722 2012), was attenuated when exercise was performed in a training-accustomed state (Fernandez-
723 Gonzalo *et al.*, 2013). Together, these data lend support to the notion the molecular signals
724 initiated in skeletal muscle by exercise become more mode-specific with repeated training, and
725 increases in post-exercise mTORC1 signalling with concurrent exercise may be attenuated
726 when performed in a training-accustomed state.
727
728 While the observed mTORC1 signalling responses were consistent with the paradigm of
729 enhanced mode-specificity of molecular responses with repeated training, the finding of greater
730 AMPK phosphorylation following RE compared with concurrent exercise was unexpected
731 given the energy-sensing nature of AMPK signalling and its purported role in promoting an
732 oxidative skeletal muscle phenotype (McGee & Hargreaves, 2010). This observation may
733 suggest an adaptive response whereby endurance training rendered subjects in the concurrent
734 training groups less susceptible to exercise-induced metabolic perturbation in skeletal muscle,
735 manifesting in an attenuated post-exercise AMPK phosphorylation response. A similar

phenomenon has been observed in human skeletal muscle after only 10 days of endurance training, whereby post-exercise increases in AMPK activity following a single pre-training exercise bout are attenuated compared with the same exercise bout performed before training (McConell *et al.*, 2005). The present data suggest further work is required to further define the mode-specificity of AMPK signalling in skeletal muscle and the effect of repeated training on the induction of these responses.

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

761 may be similarly attenuated when RE is combined with endurance exercise in the form of either
762 HIT or MICT.
763
764 The regulation of several Pol-I associated proteins was also measured at the transcriptional
765 level, including TIF-1A, POLR1B, UBF, and cyclin D1. Concurrent exercise, irrespective of
766 endurance training intensity, was a sufficient stimulus for increasing POLR1B mRNA
767 expression at 3 h post-exercise, but only MICT+RT and RT alone increased TIF-IA mRNA
768 content at this timepoint. Previous work in human skeletal muscle has demonstrated no effect
769 of a single session of RE performed in either untrained or trained states on the mRNA
770 expression of either TIF-1A or POLR1B at either 1 h (Figueiredo *et al.*, 2015) or 4 h (Nader *et*
771 *al.*, 2014) post-exercise. Eight weeks of RT has previously been shown to increase basal UBF
772 mRNA expression, which was reduced 1 h following a single session of RE performed post-
773 training (Figueiredo *et al.*, 2015). Although there were no basal training-induced increases in
774 UBF mRNA expression for any training group in the present study, a similar reduction in UBF
775 mRNA content was noted 3 h post-exercise for the RT group. Increased cyclin D1 mRNA was
776 also seen at rest post-training for the HIT+RT group, which was maintained at 3 h post-
777 exercise. Figueiredo et al. (2015) have shown eight weeks of RT decreased post-training levels
778 of cyclin D1 mRNA compared with pre-training, with a small increase induced at 1 h post-
779 exercise by a single session of post-training RE. It therefore appears HIT is a more potent
780 stimulus for increasing levels of cyclin D1 mRNA compared with RE alone or MICT, although
781 an acute reduction in cyclin D1 protein levels was also seen 1 h following a single bout of
782 HIT+RT. Previous work has shown increases in cyclin D1 mRNA during long-term (3 months)
783 RT (Kadi *et al.*, 2004), which may suggest an increase in satellite cell activation and
784 proliferation during the training intervention (Adams *et al.*, 1999; Kadi *et al.*, 2004), although
785 direct measures of these markers were not made in the present study.

786

787 Despite the present findings regarding signalling responses upstream of 45S pre-rRNA
788 transcription, the expression of 45S pre-RNA, but not mature ribosome species, was increased
789 only by a single session of concurrent exercise and not by RE alone. Previous work in humans
790 has reported basal increases in 45S pre-rRNA after 8 weeks of RT (Figueiredo *et al.*, 2015),
791 and 4 h after a single session of RE performed in both untrained and trained states (Nader *et*
792 *al.*, 2014). Notably, post-exercise expression of 45S pre-rRNA was less pronounced in the
793 trained compared with untrained state (Nader *et al.*, 2014). While no substantial basal changes
794 in 45S pre-rRNA expression were observed in the present study, the change in 45S pre-rRNA
795 levels between PRE-T and POST-T was greater for both concurrent training groups compared
796 with RT performed alone. Concurrent exercise also increased 45S pre-rRNA levels at 3 h post-
797 exercise, with little effect of single-mode RE. These observations may be explained by the
798 muscle sampling timepoints employed in the present study. Increased post-exercise 45S pre-
799 rRNA levels have been previously shown at a later timepoint of 4 h after RE (Nader *et al.*,
800 2014), whereas a reduction in 45S rRNA levels has been demonstrated 1 h post-RE in trained,
801 but not untrained, states (Figueiredo *et al.*, 2015). The possibility therefore exists that RE may
802 increase 45S rRNA expression at a later timepoint post-exercise, and the sampling time points
803 employed herein were not extensive enough to measure any exercise-induced increases in 45S
804 pre-rRNA expression.

805

806 The effects of training on the basal expression of mature ribosome species 5.8S, 18S, and 28S
807 were also investigated, as well as early post-exercise changes in mature rRNA expression.
808 Contrary to the a-priori hypothesis, RT induced small decreases in the levels of both the 5.8S
809 and 28S rRNAs in the basal state post-training, while the training-induced change in both of
810 these rRNAs was greater with concurrent exercise compared with RT alone. Neither training

811 protocol induced any changes in 18S rRNA expression. Previous work has observed basal
812 increases in 5.8S, 18S, and 28S rRNA expression in human skeletal muscle after 8 weeks of
813 RT, all of which were reduced 1 h following a single session of RE performed post-training
814 (Figueiredo *et al.*, 2015). The present data contrast with these findings by suggesting that in
815 parallel with training-induced changes in total RNA content, RT performed alone was an
816 insufficient stimulus to increase mature rRNA content, whereas concurrent exercise was
817 sufficient to increase mature 5.8S and 28S expression after a single post-training exercise
818 session.
819
820 The rRNA primers used in the present study were specifically designed to differentiate between
821 mature rRNA expression and the expression of these sequences when still bound to the
822 polycistrionic 45S rRNA precursor (i.e., 5.8S, 18S and 28S [span] rRNA) (Figueiredo *et al.*,
823 2015). Using identical primers as the present study, previous work has shown basal training-
824 induced increases in mature rRNA expression did not occur concomitantly with likewise
825 increased expression of rRNA transcripts still bound to the 45S precursor (i.e., 5.8S, 18S and
826 28S [span]), suggesting a training-induced increase in mature rRNA content, rather than simply
827 increased 45S precursor expression (Figueiredo *et al.*, 2015). In contrast, we observed
828 simultaneous post-exercise increases in the expression of both mature rRNA transcripts and
829 those still bound to the 45S precursor (i.e., 'span' rRNA transcripts). It is therefore possible
830 our observed changes in these markers may be reflective solely of changes in 45S pre-rRNA
831 content, and not the mature forms of these rRNAs. However, it is also possible this may relate
832 to the post-exercise time course examined in the present study. In support of this notion, it was
833 shown that a single session of RE was sufficient to increase only the expression of rRNA
834 transcripts still bound to the 45S pre-rRNA, and not mature rRNA species, even after 48 h of
835 post-exercise recovery (Figueiredo *et al.*, 2016). It is therefore plausible that the post-exercise

836 time courses examined in the present study were not extensive enough to measure early post-
837 exercise changes in mature rRNA expression. Clearly, further work is required to investigate
838 the time course of rRNA regulation with training in human skeletal muscle.
839
840 Consistent with the training-induced changes in both 5.8S and 28S rRNA expression with RT
841 performed alone, we observed a small reduction in basal total RNA content in skeletal muscle
842 within this cohort. Despite this paradoxical finding, it is interesting to note total RNA content
843 was higher at PRE-T for the RT group compared with both the HIT+RT and MICT groups
844 (1.6- and 1.3-fold, respectively). The reason for this between-group discrepancy at baseline is
845 not immediately clear, given we previously showed no differences in baseline lean mass
846 measured via DXA or lower-body 1-RM strength in these participants (Fyfe *et al.*, 2016a),
847 suggesting other factors may have influenced the between-group differences in baseline
848 skeletal muscle RNA content. It is also possible that the training program provided an
849 insufficient stimulus to at least maintain this elevated basal RNA content for the RT group.
850 Studies demonstrating robust increases in total RNA content concomitantly with rodent
851 skeletal muscle hypertrophy typically employ supraphysiological methods for inducing muscle
852 hypertrophy, such as synergist ablation (Goodman *et al.*, 2011b; Miyazaki *et al.*, 2011; von
853 Walden *et al.*, 2012; Nakada *et al.*, 2016), a stimulus clearly not replicated by RT in human
854 models. Participant training status may also impact upon training-induced changes in ribosome
855 biogenesis in humans. The participants in the present study were actively engaging in resistance
856 and/or endurance exercise for at least 1 year prior to commencing the study, suggesting a higher
857 relative training status compared with those of Figueiredo et al. (2015) (although this was not
858 made explicitly clear, and participants were asked to refrain from RT for 3 weeks prior to the
859 study). It is also possible that between-group differences in training volume, which was clearly

higher for the concurrent training groups compared with RT, may have impacted upon the training-induced changes in total skeletal muscle RNA content.

Despite the changes in skeletal muscle RNA content, RT was sufficient to increase type I muscle fibre CSA. In agreement with previous research (Kraemer *et al.*, 1995; Bell *et al.*, 2000), the training-induced increase in type I muscle fibre CSA was attenuated with concurrent exercise, albeit only when incorporating HIT, compared with RT performed alone. Despite these between-group differences in fibre-type specific hypertrophy, we could find no evidence that the training-induced changes in lean mass or muscle fibre CSA were correlated with changes in total RNA content of skeletal muscle (data not shown). The apparent disconnect between training-induced changes in total RNA content and markers of muscle hypertrophy, both at the whole-body and muscle-fibre levels, suggests further investigation is required into relationship between changes in translational capacity and RT-induced hypertrophy in human skeletal muscle.

As skeletal muscle mass accretion is ultimately determined by the net balance between MPS and protein degradation (Atherton & Smith, 2012), the expression of ubiquitin ligases purported to mediate muscle protein breakdown (Bodine *et al.*, 2001a) was also measured as proxy markers of protein degradation. Concurrent exercise incorporating HIT has previously been shown to exacerbate the expression of MuRF-1 relative to RE performed alone (Apro *et al.*, 2015), while we previously reported similar increases in MuRF-1 mRNA expression 3 h after a single bout of concurrent exercise incorporating either HIT or MICT in relatively training-unaccustomed individuals (Fyfe *et al.*, 2016b). Conversely, when performed in the trained state, the present data suggest only the HIT protocol was sufficient to induce elevated MuRF-1 expression after subsequent RE, relative to RE either performed alone or in

combination with MICT. While the role of Atrogin-1 in mediating protein degradation is less clear compared with MuRF-1 (Krawiec *et al.*, 2005), we nevertheless observed a reduction in Atrogin-1 expression at +3 h for RE, but not for either concurrent exercise group. These data are consistent with previous reports of reduced Atrogin-1 expression 3 h after RE performed in both untrained and trained states (Fernandez-Gonzalo *et al.*, 2013), but contrast others showing reduced Atrogin-1 expression 3 h after RE only when preceded 6 h earlier by MICT (40 min cycling at 70% of peak power output) (Lundberg *et al.*, 2012). Taken together, these data suggest concurrent exercise incorporating HIT may exacerbate post-exercise rates of protein degradation by increasing MuRF-1 mRNA expression, while both concurrent exercise protocols prevented the acute reduction in Atrogin-1 expression induced by RE alone. These data should, however, be considered with recent evidence suggesting increased rates of protein degradation may be necessary to promote skeletal muscle remodelling and be permissive, rather than inhibitory, for training adaptations in skeletal muscle (Vainshtein & Hood, 2015).

9. Conclusions

This is the first study to simultaneously investigate markers of ribosome biogenesis and mTORC1 signalling in human skeletal muscle following concurrent training compared with RT performed alone. Contrary to our hypotheses, and recent work in humans (Nader *et al.*, 2014; Figueiredo *et al.*, 2015), we noted little evidence of ribosome biogenesis in skeletal muscle following eight weeks of RT. Rather, increases in markers of ribosome biogenesis, albeit small in magnitude, tended to be greater following concurrent exercise and were independent of the endurance training intensity employed. This occurred despite a single session of RE being a more potent stimulus for both mTORC1 signalling and phosphorylation of regulators of RNA Pol-1-mediated rDNA transcription (i.e., TIF-1A and UBF) when performed post-training. An apparent disconnect was noted between training-induced changes

910 in muscle fibre CSA, of which the small increases induced by RT were attenuated when
911 combined with HIT, and changes in total skeletal muscle RNA content. Overall, the present
912 data suggest single-mode RE performed in a training-accustomed state preferentially induces
913 mTORC1 and ribosome biogenesis-related signalling in skeletal muscle compared with
914 concurrent exercise; however, this is not associated with basal post-training increases in
915 markers of ribosome biogenesis. The observation that both mTORC1 and ribosome biogenesis-
916 related signalling were impaired in response to the final training session of the study for both
917 HIT+RT and MICT+RT, relative to RE performed alone, suggests RT may become a greater
918 stimulus for ribosome biogenesis and muscle hypertrophy if training were continued long-term.
919 Further work in human exercise models that stimulate more robust skeletal muscle hypertrophy
920 (e.g., high-volume RT performed to failure), together with longer training periods, may be
921 required to further elucidate the role of ribosome biogenesis in adaptation to RT, and
922 subsequently any potential interference to these responses with concurrent training.
923

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11. Additional information section

Competing interests

The authors declare no conflicts of interest relevant to the contents of this manuscript.

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Author contributions

- Study design was performed by J.J.F., J.D.B., E.D.H., D.J.B. and N.K.S. Data collection was performed by J.J.F, M.J.A and A.P.G. Analysis and interpretation of data was performed by J.J.F., J.D.B., E.D.H., D.J.B. and N.K.S. The manuscript was written by J.J.F., D.J.B., and N.K.S. and J.D.B., E.D.H., M.J.A and A.P.G. critically revised the manuscript. All authors approved the final version of the manuscript.
- All data collection and data analysis for this study was conducted and performed in the exercise physiology and biochemistry laboratories at Victoria University, Footscray Park campus, Melbourne Australia.
- All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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12. Authors' translational perspective

Optimising adaptations to divergent exercise modes (i.e., resistance and endurance training) is important for maximising the associated health and exercise performance benefits. Concurrent training (i.e., combined resistance and endurance training) can result in compromised development of strength and muscle mass compared with resistance training performed alone. Despite this, the molecular mechanisms that underpin this altered adaptation to concurrent training in skeletal muscle are unclear. Recent evidence suggests ribosome biogenesis plays an important role in skeletal muscle hypertrophy; however, whether altered ribosome biogenesis occurs in human skeletal muscle with concurrent training is unclear. The present study has shown that ribosome biogenesis adaptation is not compromised following short-term concurrent training, despite attenuated signalling responses in skeletal muscle related to both ribosome biogenesis and translational efficiency (i.e., mTORC1 signalling) following a single session of concurrent exercise performed in a training-accustomed state. Importantly, compromised mTORC1 signalling does not appear to be evident when exercise is performed in untrained individuals, or in those not accustomed to the exercise protocol. The results of this study suggest that attenuated signalling responses related to changes in translational efficiency and capacity in skeletal muscle following single bouts of concurrent exercise, performed after a period of training, do not appear to be related to basal adaptive responses to short-term concurrent training. However, it is possible that these attenuated early post-exercise responses seen following concurrent exercise may underpin blunted adaptation to longer-term concurrent training.

13. Tables

Table 1. Details of PCR primers used for mRNA analysis

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

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

Table 2. Details of PCR primers used for rRNA analysis

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

Table 3. Physiological and psychological (RPE) responses to a single bout of high-intensity interval training (HIT) or work-matched moderate-intensity continuous training (MICT) performed during the final training session.

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

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

Table 4. Venous blood lactate and glucose responses to a single bout of resistance exercise (RE) either performed alone (RT) or when performed after either high-intensity interval training (HIT+RT) or work-matched moderate-intensity continuous training (MICT+RT) during the final training session.

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

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

Table 5. Total RNA content and type I and type II muscle fibre cross-sectional area (CSA) of the vastus lateralis before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT).

Measure		PRE-T	POST-T
Total skeletal muscle RNA (ng/mg tissue)			
	RT	914 ± 202^	810 ± 134*
	HIT+RT	581 ± 176	740 ± 129
	MICT+RT	680 ± 81	818 ± 133
Type I muscle fibre CSA (μm^2)			
	RT	4539 ± 848	5533 ± 1913*[b]
	HIT+RT	6713 ± 1849	5183 ± 1413
	MICT+RT	5509 ± 2326	5228 ± 1277
Type II muscle fibre CSA (μm^2)			
	RT	5296 ± 1347	6456 ± 2235
	HIT+RT	6470 ± 1481	6621 ± 2018
	MICT+RT	5051 ± 1531	5728 ± 688

Data presented are means ± SD. * = $P < 0.05$ vs. PRE-T, ^ = $P < 0.05$ vs. both HIT+RT and MICT+RT at PRE-T, b = change between PRE-T and POST-T substantially greater vs. HIT+RT.

14. Figure and legends

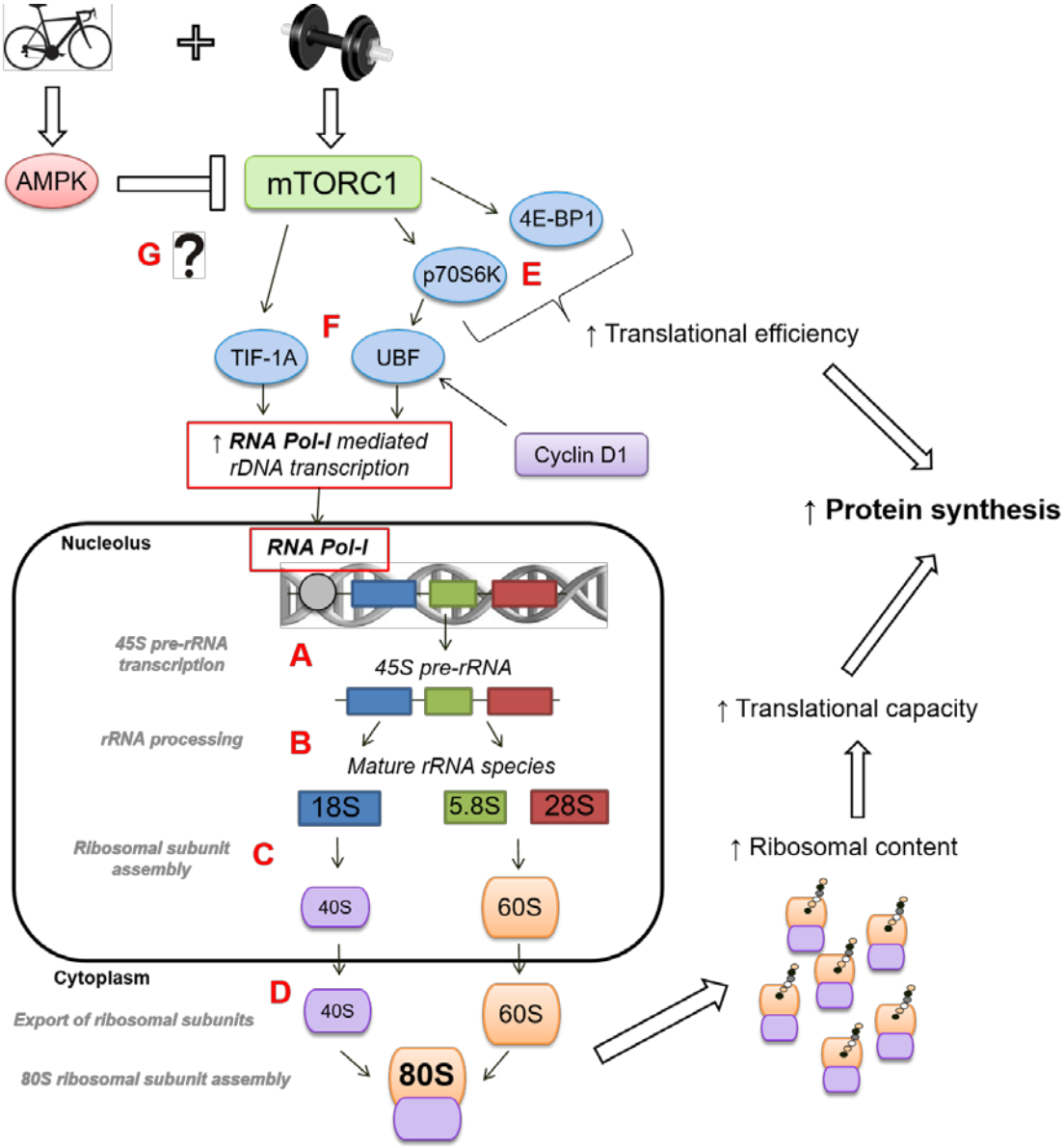

Figure 1. Overview of the role of mTORC1 signalling in promoting ribosome biogenesis following a single session of resistance exercise, and the potential effect of incorporating endurance training (i.e, performing concurrent training). Adapted from (Chaillou *et al.*, 2014). Ribosome biogenesis involves transcription of the 45S rRNA (ribosomal RNA) precursor (45S pre-rRNA) **(A)** mediated by RNA Polymerase I (Pol-I), processing of the 45S pre-rRNA into several smaller rRNAs (18S, 5.8S and 28S rRNAs) **(B)**, assembly of these rRNAs and other ribosomal proteins into ribosomal subunits (40S and 60S) **(C)**, and nuclear export of these ribosomal subunits into the cytoplasm (Thomson *et al.*, 2013; Chaillou *et al.*, 2014) **(D)**.

As well as regulating translational efficiency via downstream control of p70S6K (p70 kDa ribosomal protein subunit kinase 1) and 4E-BP1 (eukaryotic initiation factor 4E binding protein 1) **(E)**, mTORC1 is a key mediator of ribosome biogenesis by regulating transcription factors for genes encoding RNA Pol-I (and also RNA Pol-II and –III, which are not shown in figure) (Iadevaia *et al.*, 2014). Transcription of the 45S pre-rRNA by RNA Pol-I requires a transcriptional complex including TIF-1A (transcription initiation factor 1A; also known as RRN5) and UBF (upstream binding factor), both of which are regulated by the mTORC1 pathway (Hannan *et al.*, 2003; Mayer *et al.*, 2004) **(F)**.

Activation of AMPK is known to inhibit mTORC1 signalling in rodent skeletal muscle (Thomson *et al.*, 2008), and AMPK activation in skeletal muscle is traditionally associated with endurance-type exercise. However, whether signalling events initiated by endurance training, when performed concurrently with resistance training, have the potential to interfere with mTORC1-mediated regulation of ribosome biogenesis is currently unclear **(G)**.

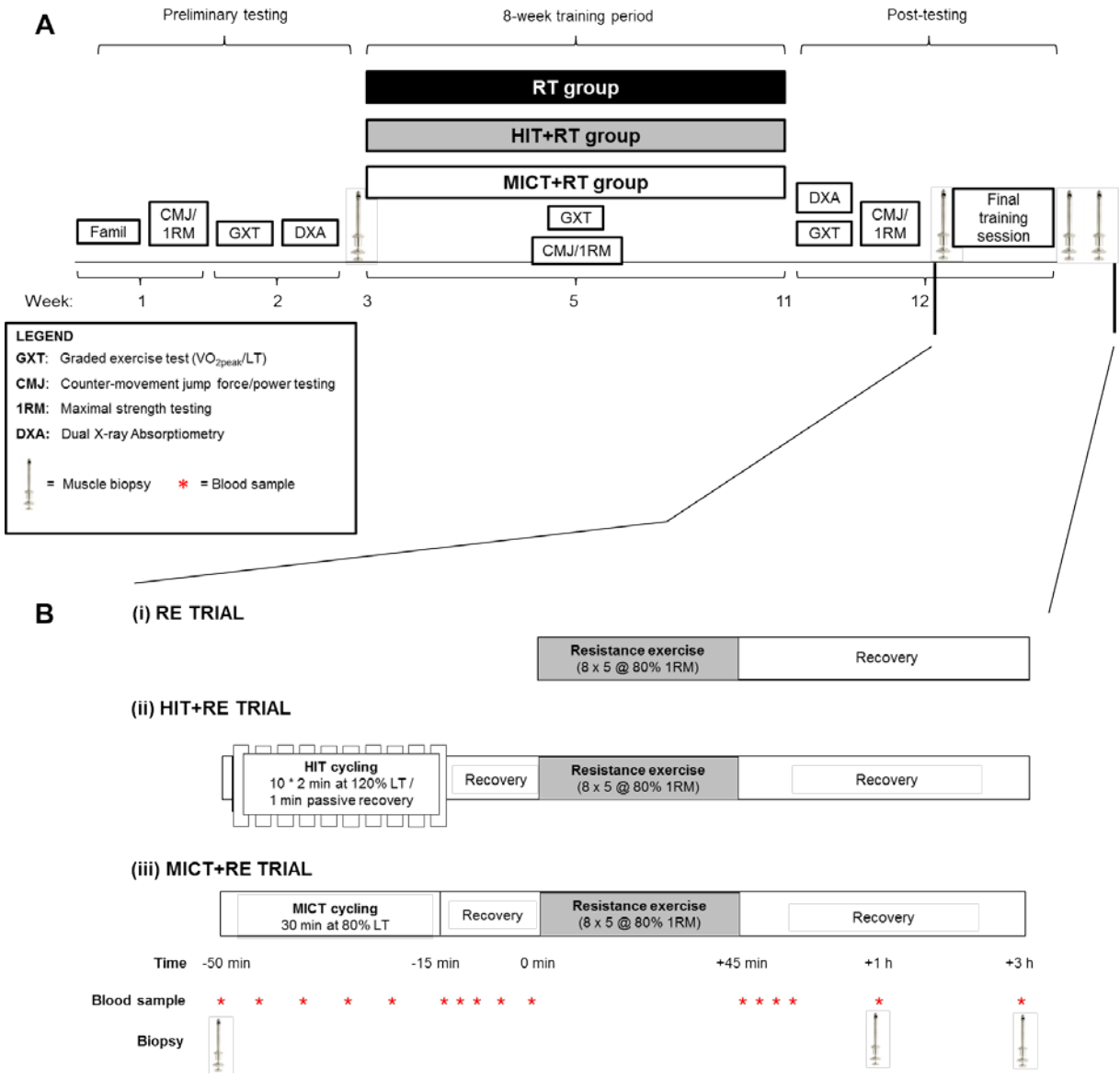

Figure 2. Study overview (A) and timelines for the final training session (B). Participants first completed 8 weeks of either resistance training (RT) alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT). For the final training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

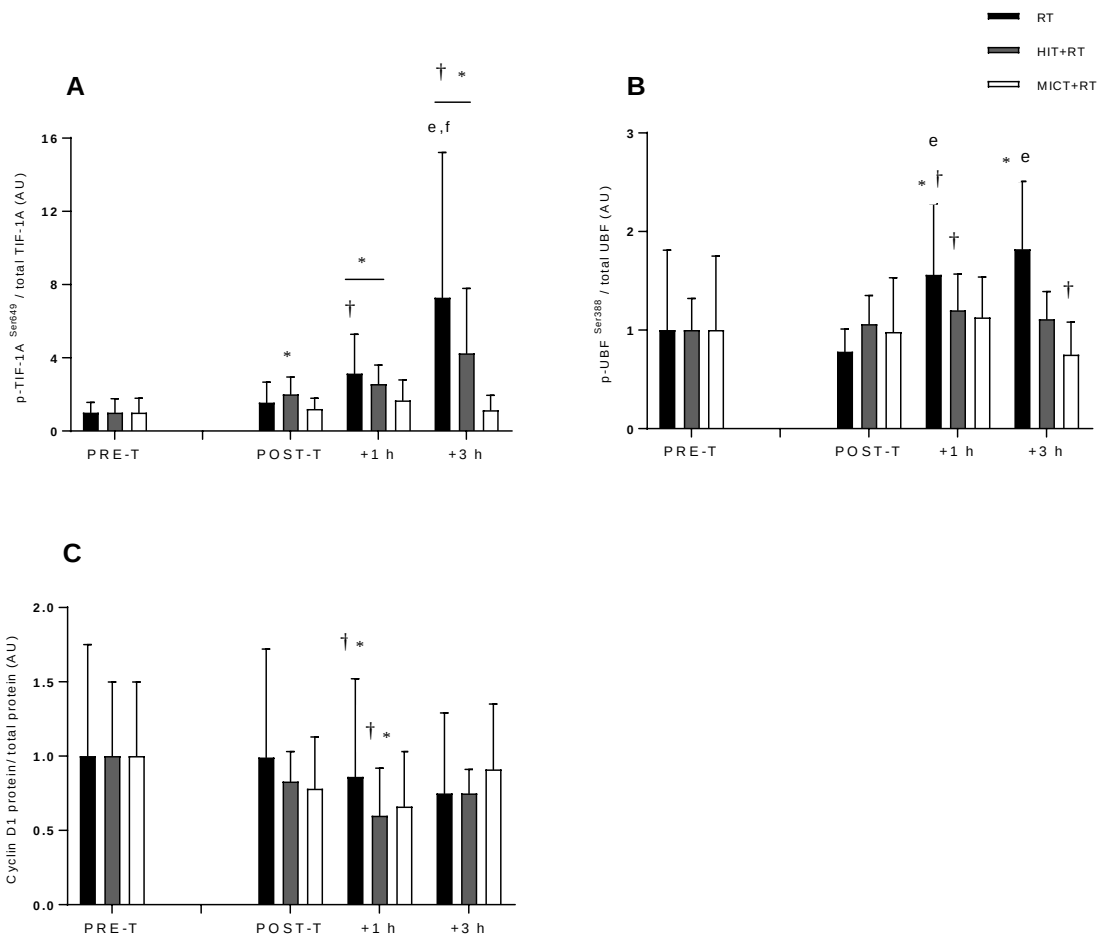

Figure 3. Phosphorylation of TIF-1A^{Ser649} (A), UBF^{Ser388} (B), and total protein content of cyclin D1 (C) before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and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

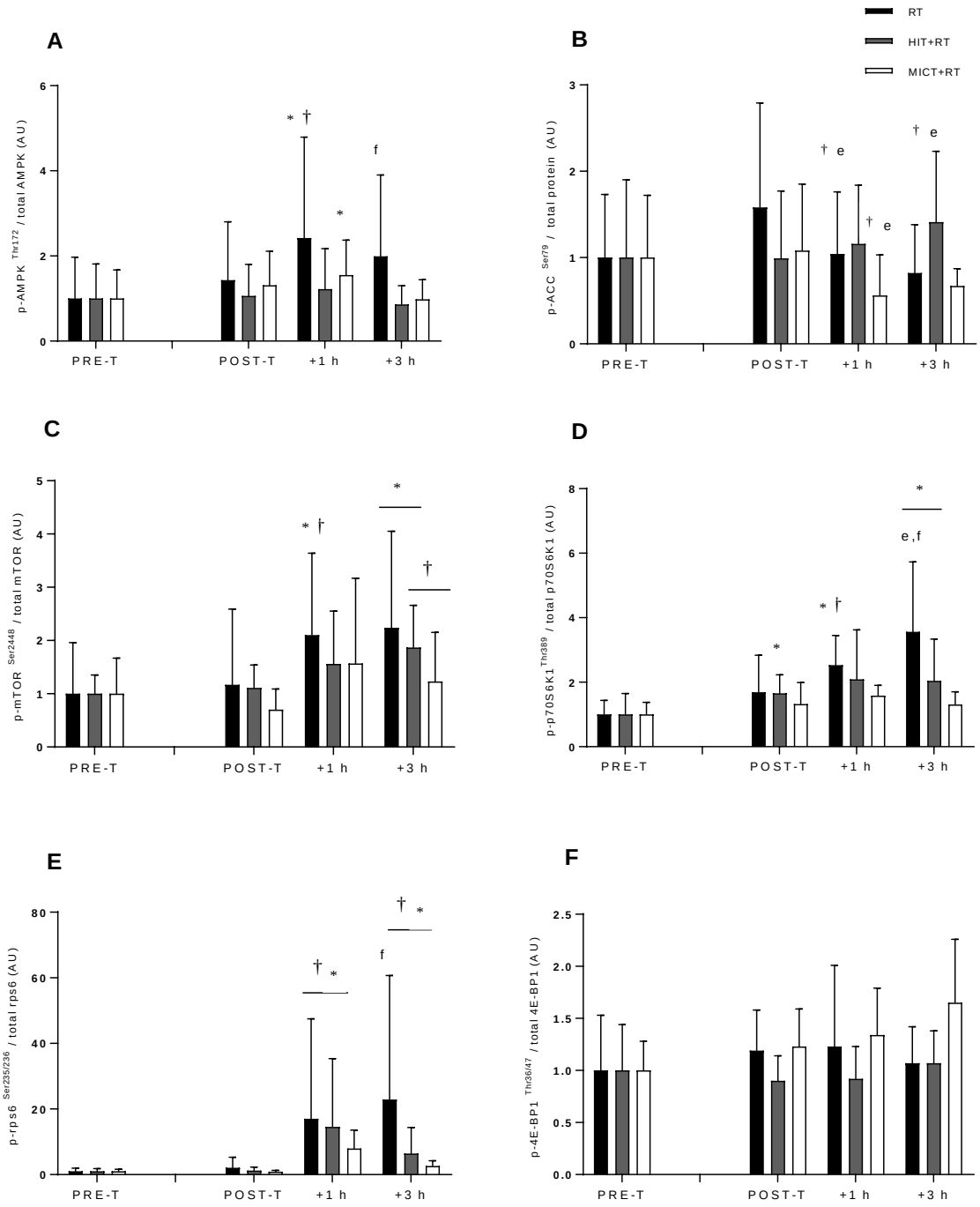

Figure 4. Phosphorylation of AMPK^{Thr172} (A), ACC^{Ser79} (B), mTOR^{Ser2448} (C), p70S6K^{Thr389} (D), rps6$^{Ser235/236}$ (E) and 4E-BP1$^{Thr36/47}$ (F) before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for each corresponding group. * = $P < 0.05$ vs. PRE-T, † = $P < 0.05$ vs. POST-T. Change from POST-T substantially greater vs. e = HIT+RT, f = MICT+RT.

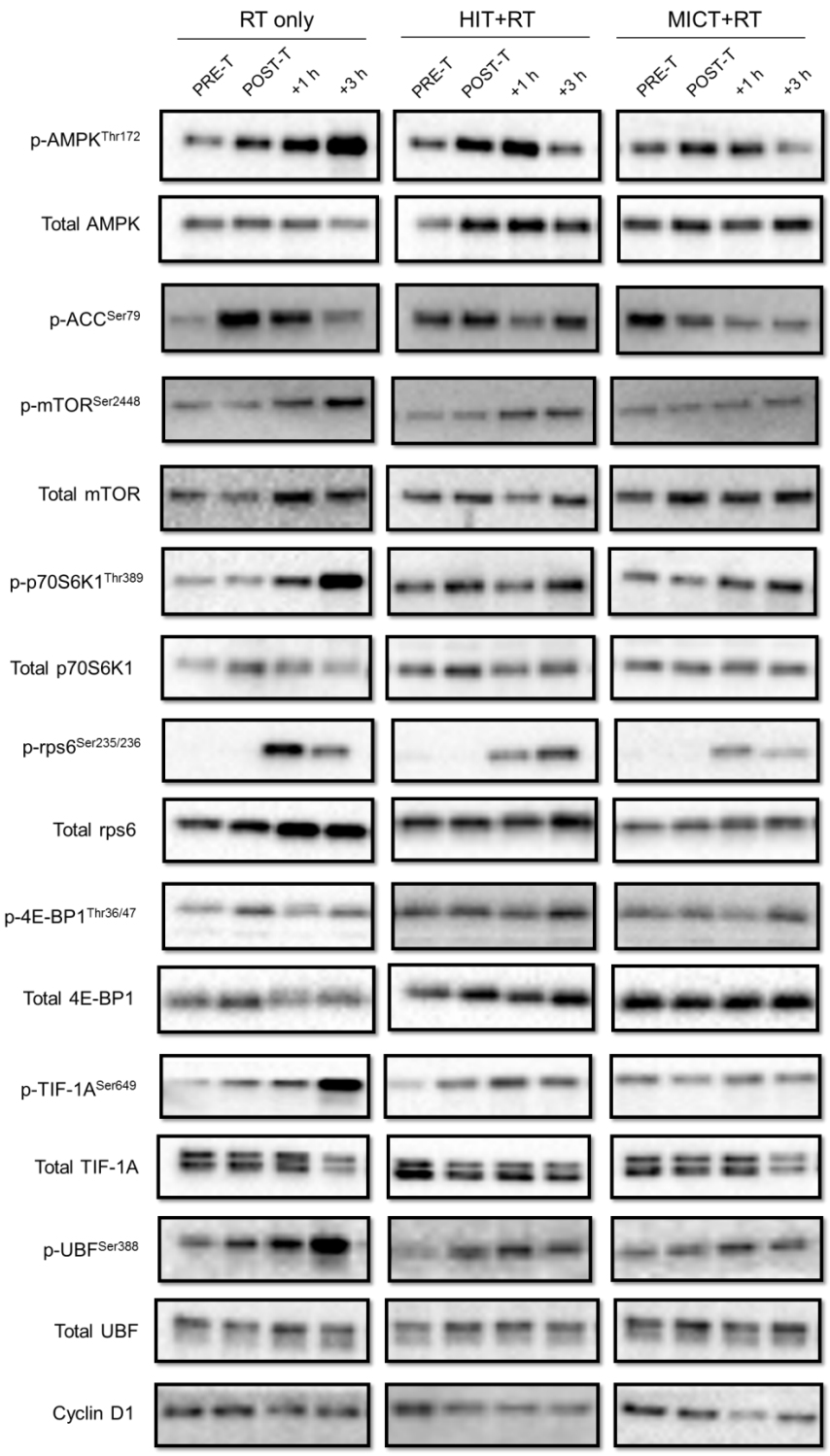

Figure 5. Representative western blots for the phosphorylation (p-) and total protein content of signalling proteins before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h (+1 h) and 3 h (+3 h) after a single exercise bout performed post-training.

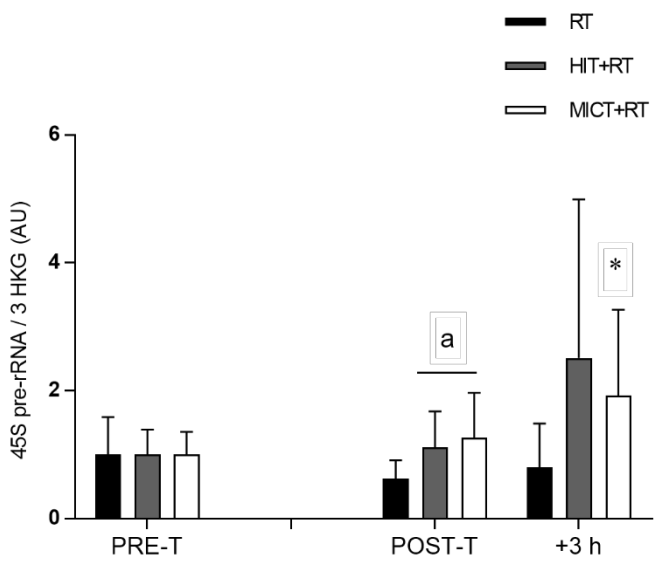

Figure 6. Expression of 45S pre-rRNA relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE-T value for each corresponding group. * = $P < 0.05$ vs. PRE-T, a = change between PRE-T and POST-T substantially different vs. RT.

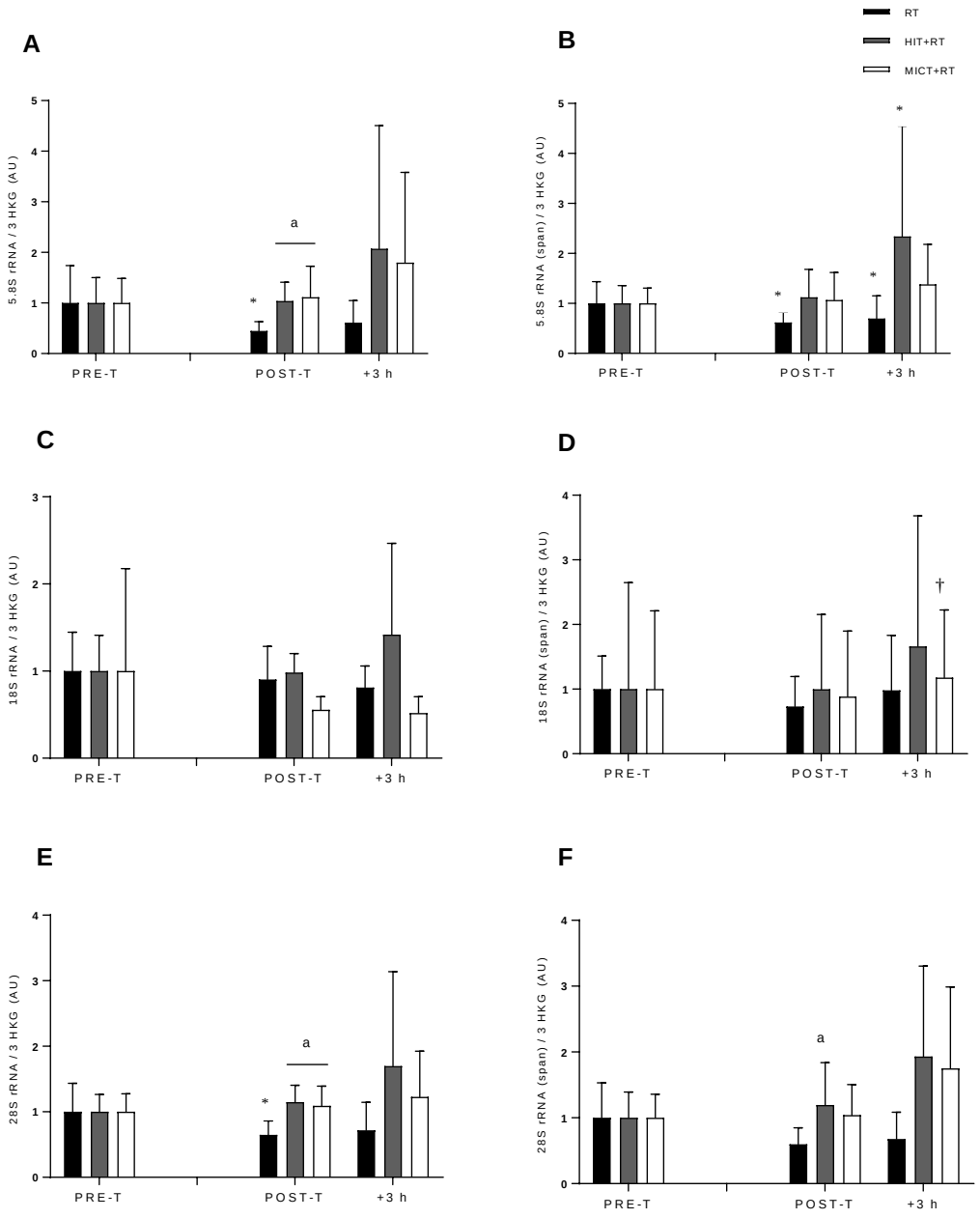

Figure 7. Expression of the mature rRNA transcripts 5.8S rRNA (A), 18S rRNA (C), and 28S rRNA (E), and rRNA transcripts bound to the 45S pre-RNA precursor: 5.8S rRNA (span) (B) 18S rRNA (span) (D) and 28S rRNA (span) (F) relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE-T value for each corresponding group. * = $P < 0.05$ vs. PRE-T, † = $P < 0.05$ vs. POST-T, a = change between PRE-T and POST-T substantially greater vs RT.

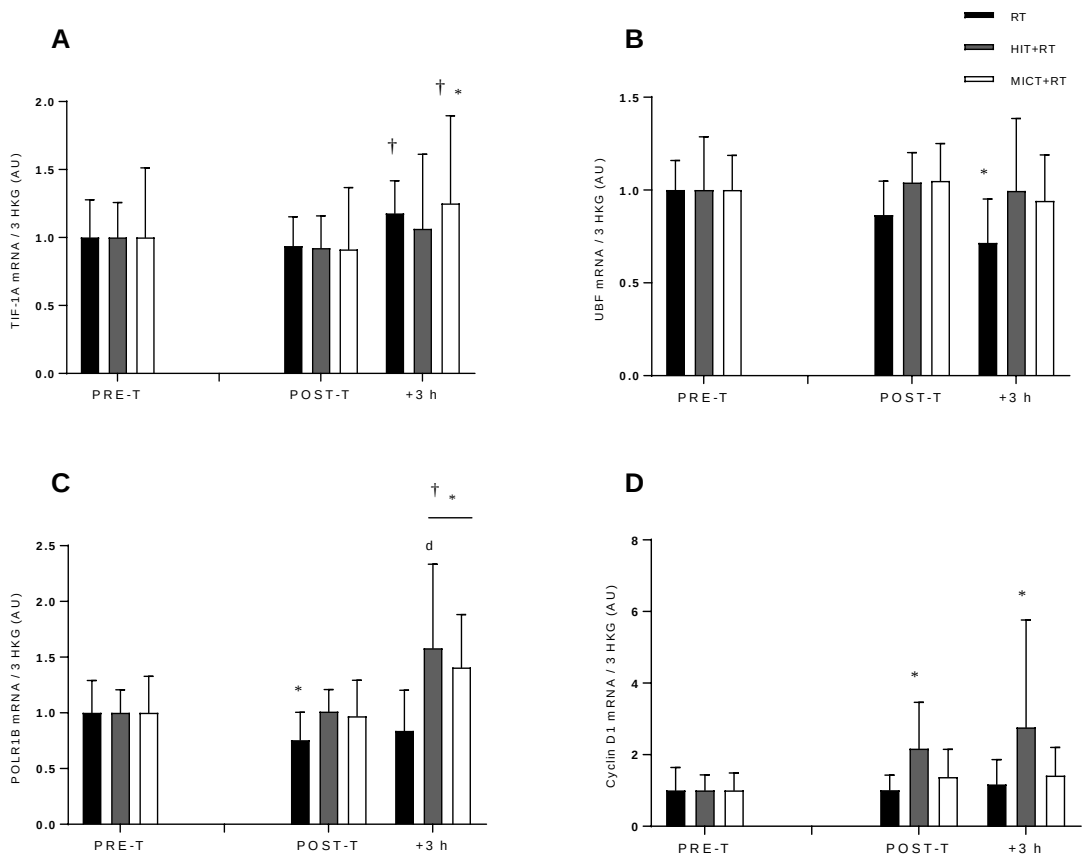

Figure 8. mRNA expression of TIF-1A (A), UBF (B), POLR1B (C), and cyclin D1 (D) relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for each corresponding group. * = $P < 0.05$ vs. PRE, † = $P < 0.05$ vs. POST. Change from POST substantially greater vs. d = RT.

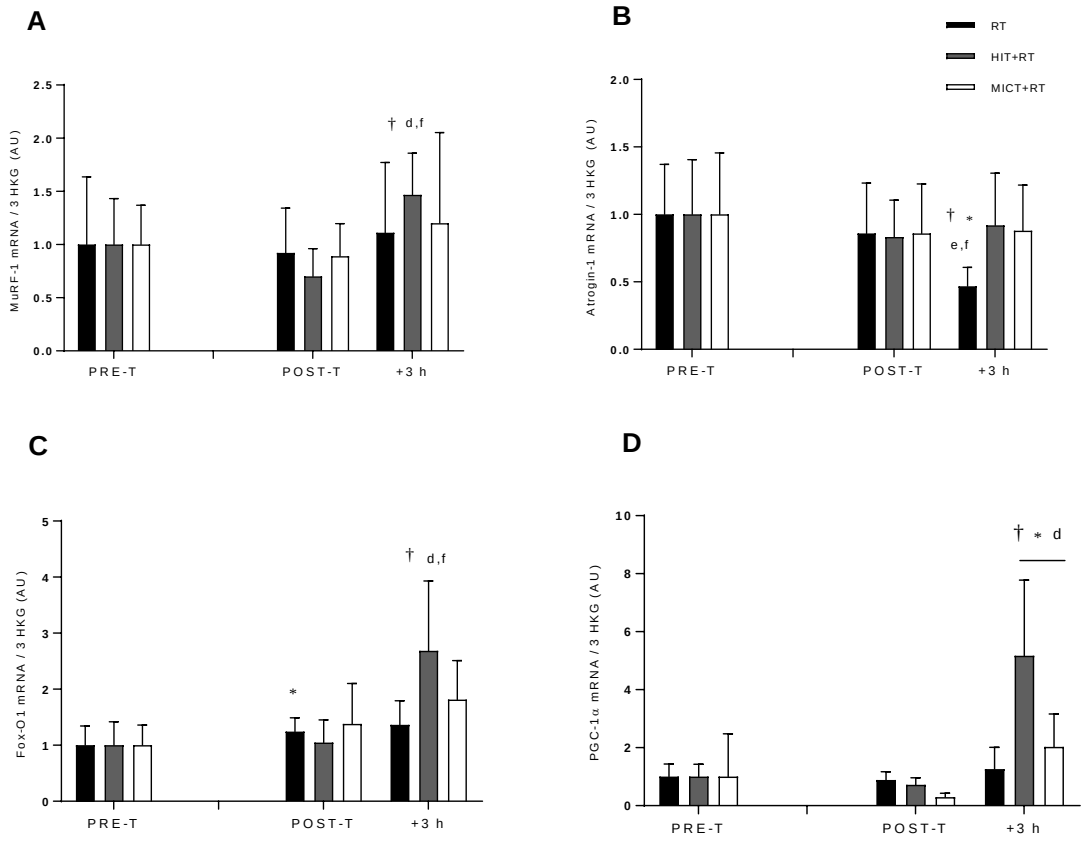

Figure 9. mRNA expression of MuRF-1 (A), Atrogin-1 (B), Fox-O1 (C) and PGC-1α (D) relative to the geometric mean of cyclophillin, β2M and TBP expression before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means ± SD and expressed relative to the PRE value for each corresponding group. * = $P < 0.05$ vs. PRE, † = $P < 0.05$ vs. POST. Change from POST substantially greater vs. d = RT, e = HIT+RT, f = MICT+RT.

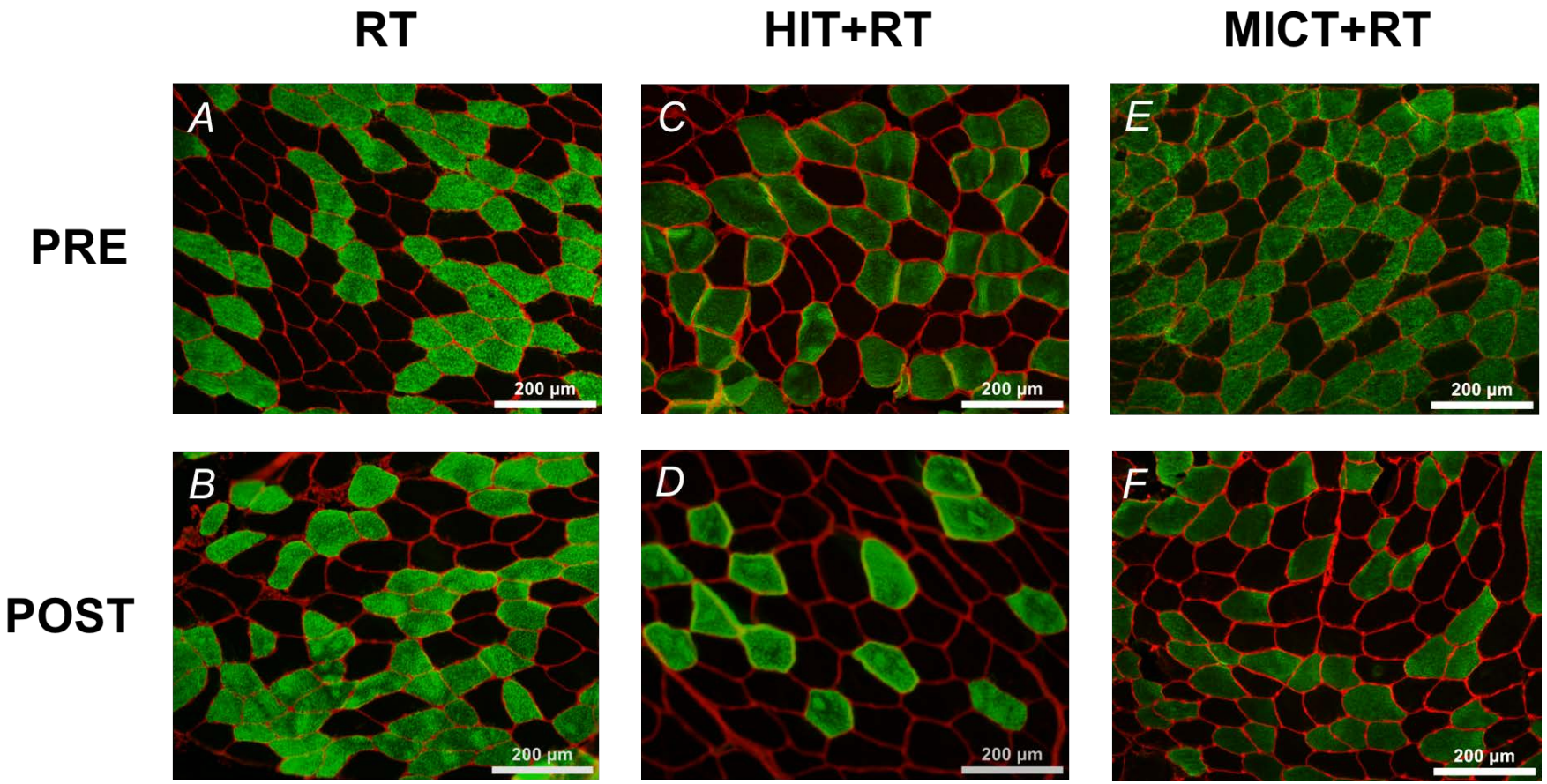

Figure 10. Representative immunohistochemical images of muscle cross-sections obtained before (PRE) and after (POST) eight weeks of either RT alone (images A and B, respectively), or RT combined with either high-intensity interval training (HIT+RT; images C and D, respectively) or moderate-intensity continuous training (MICT+RT; images E and F, respectively). Muscle fibre membranes are stained red, type I muscle fibres are stained green, and type II muscle fibres are unstained.

15. Supplementary material

Supplementary table 1. Summary of magnitude-based inference (MBI) data for all within-group comparisons.

Supplementary table 2. Summary of magnitude-based inference (MBI) data for all between-group comparisons.