

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

3

4 **Authors:** Jackson J. Fyfe^{1,2,3}, David J. Bishop^{1,4}, Jonathan D. Bartlett¹, Erik D. Hanson^{1,5}, Mitchell J.
5 Anderson¹, Andrew P. Garnham^{1,2} & Nigel K. Stepto^{1,6,7}.

6

7 **Affiliations:**

8 1) Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Melbourne, Australia;
9 2) School of Exercise and Nutrition Sciences, Deakin University, Melbourne, Australia; 3) Institute
10 for Physical Activity and Nutrition (IPAN), Deakin University, Melbourne, Australia; 4) School of
11 Medical and Health Sciences, Edith Cowan University, Joondalup, Australia; 5) Department of
12 Exercise and Sport Science, University of North Carolina at Chapel Hill, North Carolina, USA; 6)
13 Monash Centre for Health Research and Implementation, School of Public Health and Preventive
14 Medicine, Monash University, Melbourne, Australia; 7) Australian Institute for Musculoskeletal
15 Science (AIMSS), University of Melbourne, Victoria University and Western Health, Sunshine
16 Hospital, St Albans, Australia

17

18 **Running head:** Concurrent training and skeletal muscle ribosome biogenesis

19

20 **Address for correspondence:**

21 Jackson J. Fyfe

22 Institute for Physical Activity and Nutrition (IPAN)

23 School of Exercise and Nutrition Sciences

24 Deakin University

25 221 Burwood Hwy, Burwood VIC 3125

26 Email: jackson.fyfe@deakin.edu.au

27

28 **Keywords:** Concurrent training, ribosome biogenesis, mTORC1 signalling.

29

30 **Table of contents category:** Muscle

31

32

33

34 **1. Key points summary**

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

50

51

52 2. Abstract

53 Combining RT with endurance training (i.e., concurrent training) may attenuate skeletal muscle
54 hypertrophy consequent to RT; however, the underlying mechanisms are unclear. We investigated
55 whether markers of ribosome biogenesis, a process linked with skeletal muscle hypertrophy, are
56 attenuated following concurrent training vs. RT alone. Twenty-three males (mean \pm SD: age, 29.6 \pm
57 5.5 y; $\dot{V}O_{2peak}$, 44 \pm 11 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) underwent 8 wk (3 sessions \cdot wk $^{-1}$) of either: 1) HIT (high-
58 intensity interval training) combined with RT (HIT+RT group, $n=8$), 2) work-matched MICT
59 (moderate-intensity continuous training) combined with RT (MICT+RT group, $n=7$), or 3) RT alone
60 (RT group, $n=8$). *Vastus lateralis* biopsies were obtained before training, and immediately before, 1 h
61 and 3 h after the final training session. Type I muscle fibre cross-sectional area (CSA) was further
62 increased by RT vs. HIT+RT (34 \pm 22%; ES, 1.03 \pm 0.80), but not vs. MICT+RT (15 \pm 54%; ES, 0.39
63 \pm 1.45). Basal training-induced changes in expression of the 45S ribosomal RNA (rRNA) precursor,
64 and 5.8S and 28S mature rRNAs were greater for concurrent exercise vs. RT, largely because of trends
65 for reduced rRNA expression following RT. During the final training session, RT further increased
66 skeletal muscle mTORC1 signalling (p70S6K1 and rps6 phosphorylation) and signalling related to
67 45S rRNA transcription (TIF-1A and UBF phosphorylation) vs. concurrent exercise. Thus, when
68 performed in a training-accustomed state, RT preferentially induces mTORC1 and ribosome
69 biogenesis-related signalling in human skeletal muscle vs. concurrent exercise. However, changes in
70 markers of skeletal muscle ribosome biogenesis were more favourable with concurrent training vs.
71 RT.

72

73 **3. Abbreviations list**

74 1-RM, one-repetition maximum; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; AMPK, 5'
75 adenosine monophosphate-activated protein kinase; β 2M, beta-2 microglobulin; CDK, cyclin-
76 dependent kinase; DXA, dual-energy x-ray absorptiometry; Fox-O1, forkhead box-O1; GAPDH,
77 glyceraldehyde 3-phosphate dehydrogenase; HIT, high-intensity interval training cycling; LT, lactate
78 threshold; MICT, moderate-intensity continuous cycling; MPS, muscle protein synthesis; mRNA,
79 messenger RNA; mTORC1, mechanistic target or rapamycin complex 1; MuRF-1, muscle RING-
80 finger 1; p70S6K1, 70 kilodalton ribosomal protein subunit kinase 1; PGC-1 α , peroxisome proliferator
81 activated receptor gamma co-activator 1 alpha; POLR1B, polymerase (RNA) 1 polypeptide B; RE,
82 resistance exercise; RPE, rating of perceived exertion; rRNA, ribosomal ribonucleic acid; RT,
83 resistance training; SL-1, selectivity factor-1; TBP, TATA binding protein; TIF-1A, RRN3 polymerase
84 1 transcription factor; UBF, upstream binding factor; $\dot{V}O_{2peak}$, peak volume of oxygen uptake; W_{peak} ,
85 peak aerobic power.

86

87

88

89

90 4. Introduction

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

103
104 Subsequent work in humans (Carrithers *et al.*, 2007; Coffey *et al.*, 2009a; Coffey *et al.*, 2009b; Donges
105 *et al.*, 2012; Lundberg *et al.*, 2012; Apro *et al.*, 2013; Fernandez-Gonzalo *et al.*, 2013; Lundberg *et al.*,
106 2014; Apro *et al.*, 2015; Pugh *et al.*, 2015) has focused on the hypothesis that attenuated muscle
107 hypertrophy with concurrent training (Kraemer *et al.*, 1995; Bell *et al.*, 2000; Wilson *et al.*, 2012) may
108 be explained by AMPK-mediated inhibition of the mTORC1 pathway. Several studies, however, have
109 demonstrated that single sessions of concurrent exercise do not compromise either mTORC1 signalling
110 or rates of MPS (Carrithers *et al.*, 2007; Donges *et al.*, 2012; Apro *et al.*, 2013; Apro *et al.*, 2015; Pugh
111 *et al.*, 2015), and may even potentiate these responses (Lundberg *et al.*, 2012), compared with
112 resistance exercise (RE) performed alone. However, a limitation of these studies is that most have
113 examined these responses in either untrained individuals (Carrithers *et al.*, 2007; Donges *et al.*, 2012;
114 Pugh *et al.*, 2015) or those who are relatively unaccustomed to the exercise protocol (Lundberg *et al.*,

115 2012; Fyfe *et al.*, 2016b). Given short-term training increases the mode-specificity of post-exercise
116 molecular responses (Wilkinson *et al.*, 2008; Vissing *et al.*, 2011), examining perturbations to
117 molecular signalling and gene expression in relatively training-unaccustomed individuals may
118 confound any insight into the potential molecular mechanisms responsible for interference following
119 concurrent training (Fyfe *et al.*, 2014).

120

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

134

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

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

148

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

163

164 Studies in both human (Nader *et al.*, 2014; Figueiredo *et al.*, 2015; Stec *et al.*, 2015) and rodent skeletal
165 muscle (Adams *et al.*, 2002; Goodman *et al.*, 2011a; Miyazaki *et al.*, 2011; Chaillou *et al.*, 2012; von
166 Walden *et al.*, 2012; Chaillou *et al.*, 2013) suggest ribosome biogenesis, as indexed by increases in
167 total RNA content (>85% of which comprises rRNA) (Chaillou *et al.*, 2014), and increased mRNA
168 expression of several RNA Pol-I regulatory factors, including UBF, cyclin D1 and TIF-1A, occurs
169 concomitantly with muscle hypertrophy. In addition, attenuated rodent skeletal muscle hypertrophy
170 with ageing (Kirby *et al.*, 2015; Stec *et al.*, 2015) and rapamycin treatment (Goodman *et al.*, 2011a) is
171 associated with reduced markers of ribosome biogenesis, suggesting translational capacity is closely
172 linked to the regulation of skeletal muscle mass. Despite the links between skeletal muscle hypertrophy
173 and ribosome biogenesis (Chaillou *et al.*, 2014; Nader *et al.*, 2014; Figueiredo *et al.*, 2015), studies
174 investigating molecular interference following concurrent exercise in human skeletal muscle have only
175 measured transient (<6 h) post-exercise changes in translational efficiency (as indexed by mTORC1
176 signalling) and MPS (Carrithers *et al.*, 2007; Coffey *et al.*, 2009a; Coffey *et al.*, 2009b; Donges *et al.*,
177 2012; Lundberg *et al.*, 2012; Apro *et al.*, 2013; Fernandez-Gonzalo *et al.*, 2013; Lundberg *et al.*, 2014;
178 Apro *et al.*, 2015; Pugh *et al.*, 2015). No studies have investigated changes in ribosome biogenesis
179 either after single bouts of concurrent exercise or following periods of concurrent training. Whether
180 attenuated muscle hypertrophy following concurrent training could be explained, at least in part, by
181 attenuated ribosome biogenesis is unknown.

182
183 The aim of this study was therefore to investigate changes in markers of ribosome biogenesis and
184 mTORC1 signalling after eight weeks of concurrent training compared with RT undertaken alone. A
185 secondary aim was to determine the potential role of endurance training intensity in modulating
186 skeletal muscle ribosome biogenesis adaptation to concurrent training, by comparing concurrent
187 training incorporating either high-intensity interval training (HIT) or work-matched moderate-
188 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.

196

197 ****** INSERT FIGURE 1 ABOUT HERE ******

198

199

200 **5. Methodology**

201 *Ethical approval*

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

206

207 *Experimental overview*

208 Participant details and procedures performed in this study have been previously described (Fyfe *et al.*,
209 2016a); however, these are briefly summarised as follows. The study employed a repeated-measures,
210 parallel-group design (Figure 2A). After preliminary testing for maximal (one-repetition maximum [1-
211 RM]) strength, aerobic fitness ($\dot{V}O_{2peak}$, the lactate threshold [LT] and peak aerobic power [W_{peak}]),
212 and body composition (dual-energy x-ray absorptiometry [DXA]), participants were ranked by
213 baseline 1-RM leg press strength and randomly allocated to one of three training groups. Each group
214 performed training sessions that consisted of either 1) high-intensity interval training (HIT) cycling
215 combined with RT (HIT+RT group, $n = 8$), 2) moderate-intensity continuous training (MICT) cycling
216 combined with RT (MICT+RT group, $n = 7$) or 3) RT performed alone (RT group, $n = 8$).

217

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

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

227

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

229

230 *Training intervention*

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

236

237 *Final training session*

238 Two or three days after completion of the training intervention and post-testing, participants performed
239 a final group-specific training session (Figure 2B) whereby early post-exercise skeletal muscle
240 responses were measured in a training-accustomed state. Participants reported to the laboratory after
241 an overnight (~8-10 h) fast. After resting quietly for ~15 min upon arrival at the laboratory, a venous
242 catheter was inserted into an antecubital forearm vein and a resting blood sample was obtained. A
243 resting, post-training (POST-T) muscle biopsy was then taken from the *vastus lateralis* muscle
244 (described subsequently). Participants in the RT group waited quietly for 10 min after the POST-T
245 biopsy and then completed a standardised RT protocol (8 x 5 leg press repetitions at 80% of the post-
246 training 1RM, 3 min recovery between sets). Participants in the HIT+RT and MICT+RT groups
247 preceded the standardised RT with either HIT (10 x 2-min intervals at 140% of the post-training LT,
248 1 min passive recovery between intervals) or work- and duration-matched MICT cycling (30 min at
249 93.3% post-training LT), respectively. Fifteen minutes of passive recovery was allowed between

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

255

256 *Muscle sampling*

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

266

267 *Western blotting*

268 Approximately 5 mg of frozen muscle tissue was homogenised in lysis buffer (0.125M Tris-HCl, 4%
269 SDS, 10% Glycerol, 10mM EGTA, 0.1M DTT, 1% protease/phosphatase inhibitor cocktail), left for 1
270 h at room temperature, and then stored overnight at -80°C. The following morning, samples were
271 thawed and the protein concentration determined (Red 660 Protein Assay Kit, G-Biosciences, St.
272 Louis, MO). Bromophenol blue (0.1%) was then added to each sample, which were then stored at -
273 80°C until subsequent analysis. Proteins (8 µg) were separated by SDS-PAGE using 6-12% acrylamide
274 pre-cast gels (TGX Stain Free, Bio-Rad laboratories, Hercules, CA) in 1× running buffer (25 mM Tris,

275 192 mM Glycine, 0.1% SDS), and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-
276 Rad laboratories, Hercules, CA) using a semi-dry transfer system (Trans Blot Turbo, Bio-Rad
277 laboratories, Hercules, CA) for 7 min at 25 V. After transfer, membranes were blocked with 5% skim
278 milk in 1×TBST (200 mM Tris, 1.5 M NaCl, 0.05% Tween 20) for 1 h at room temperature, washed
279 with 1×TBST (5×5 min), and incubated with primary antibody solution (5% BSA [bovine serum
280 albumin], 0.05% Na Azide in 1×TBST) overnight at 4°C. Primary antibodies for phosphorylated (p-)
281 p-mTOR^{Ser2448} (1:1000; #5536), mTOR (1:1000), p-p70S6K1^{Thr389} (1:1000; #9234), p70S6K1
282 (1:1000), p-4E-BP1^{Thr37/46} (1:1000; #2855), 4E-BP1 (1:1000; #9452), p-AMPK^{Thr172} (1:1000; #2535),
283 AMPK (1:1000; #2532), p-rps6^{Ser235/236} (1:750; #4856), rps6 (1:1000; #2217) and p-ACC^{Ser79} (1:1000;
284 #3661) were from Cell Signalling Technology (Danvers, MA), p-UBF^{Ser388} (1:1000; sc-21637-R),
285 UBF (1:000; sc-9131) and cyclin D1 (1:1000; sc-450) were from Santa Cruz Biotechnology (Dallas,
286 TX), and p-RRN3 (TIF-1A)^{Ser649} (1:1000; ab138651) and TIF-1A (1:1000; ab70560) were from
287 Abcam (Cambridge, UK). The following morning, membranes were washed again with 1×TBST and
288 incubated with a secondary antibody (Perkin Elmer, Waltham, MA, #NEF812001EA; 1:50000 or
289 1:100000 in 5% skim milk and 1×TBST) for 1 h at room temperature. After washing again with
290 1×TBST, proteins were detected with chemiluminescence (SuperSignalTM West Femto Maximum
291 Sensitivity Substrate, Thermo Fisher Scientific, Waltham, MA) and quantified via densitometry
292 (Image Lab 5.0, Bio-Rad laboratories, Hercules, CA). All sample timepoints for each participant were
293 run on the same gel and normalised to both an internal pooled sample present on each gel, and the total
294 protein content of each lane using a stain-free imaging system (Chemi DocTM MP, Bio-Rad
295 laboratories, Hercules, CA). Phosphorylated proteins were then expressed relative to the total amount
296 of each respective protein.

297

298

299

300 *Real-time quantitative PCR (qPCR)*

301 *RNA extraction*

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

319

320 *Reverse transcription*

321 For mRNA analysis, first-strand cDNA was generated from 1 μ g RNA in 20 μ L reaction buffer using
322 the iScript[®] cDNA synthesis kit (Bio-Rad laboratories, Hercules, CA) according to manufacturer's
323 protocol, with each reaction comprising 4 μ L 5 \times iScript reaction mix, 1 μ L iScript Reverse
324 Transcriptase, 5 μ L NFW and 10 μ L of RNA sample (100 ng/ μ L). Reverse transcription was then

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

328

329 *Real-time quantitative PCR (qPCR)*

330 Real-time PCR was performed using a Realplex² PCR system (Eppendorf, Hamburg, Germany) to
331 measure mRNA levels of MuRF-1 (muscle RING-finger 1), Atrogin-1 (muscle atrophy f-box), FoxO1
332 (forkhead box-O1), PGC-1 α (peroxisome proliferator-activated gamma receptor co-activator-1 alpha),
333 UBF, TIF-1A, cyclin D1, POLR1B, and commonly used reference genes GAPDH (glyceraldehyde 3-
334 phosphate dehydrogenase), cyclophilin (also known as peptidyl-prolylcis-trans isomerase), β 2M (beta-
335 2 microglobulin) and TBP (TATA binding protein). Target rRNAs were the mature ribosome species
336 5.8S, 18S and 28S. Since primers specific for these mature rRNA sequences will also amplify pre-
337 RNA transcripts (i.e., the 45S pre-rRNA), we used specifically designed primers (QIAGEN, Venlo,
338 Limburg, The Netherlands) to distinguish between mature rRNA species and those still bound to the
339 45S pre-rRNA transcript, as previously described (Figueiredo *et al.*, 2015). Briefly, primers were
340 designed specifically for pre-rRNA sequences spanning the 5' end external/internal transcribed spacer
341 regions (ETS and ITS, respectively) of the 45S pre-RNA transcript and the internal regions of mature
342 rRNA sequences (i.e., 18S-ETS, 5.8S-ITS, and 28S-ETS). For clarity, primers amplifying the mature
343 rRNA transcripts are henceforth designated as 'mature' transcripts (e.g., 18S rRNA [mature]), as
344 opposed to those primers amplifying rRNA sequences bound to the 45S rRNA precursor, henceforth
345 designated as 'span' transcripts (e.g., 18S rRNA [span]). A specific primer for the initial region of the
346 5' end of the 45S pre-rRNA transcript was used to measure 45S pre-rRNA expression levels
347 (Figueiredo *et al.*, 2015). Standard and melting curves were performed for all primers to ensure both
348 single-product and amplification efficiency. Details for all primers used are provided in Table 1
349 (mRNA) and Table 2 (rRNA).

350

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

352

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

354

355 Each PCR reaction was performed in duplicate using a robotic pipetting machine (EpMotion 2100,
356 Eppendorf, Hamburg, Germany) in a final reaction volume of 10 μ L containing 5.0 μ L 2 \times SYBR green
357 (Bio-Rad Laboratories, Hercules, CA), 0.6 μ L PCR primers (diluted to 15 μ M; Sigma Aldrich, St.
358 Louis, MO), 0.4 μ L NFW and 4 μ L cDNA sample (diluted to 5 ng/ μ L). Conditions for the PCR
359 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
360 95°C/15 sec at 60°C, and a ramp for 20 min to 95°C. Each plate was briefly centrifuged before loading
361 into the PCR machine. To compensate for variations in input RNA amounts and efficiency of the
362 reverse transcription, mRNA data were quantified using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001)
363 and normalised to the geometric mean (Vandesompele *et al.*, 2002) of the three most stable
364 housekeeping genes analysed (cyclophilin, β 2M and TBP), determined as previously described (Mane
365 *et al.*, 2008).

366

367 *Immunohistochemistry*

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

375 (A4.840, Developmental Studies Hybridoma Bank, University of Iowa, IA), diluted 1:25 in 3%
376 BSA/PBS overnight at 4°C. The following morning, sections were washed four times in 1×PBS for 10
377 min each, before incubating with a secondary antibody (Alexa Fluor® 488 conjugate Goat anti-mouse
378 IgM, cat. no. A-21042, Thermo Fisher Scientific, Waltham, MA) diluted 1:200 in 3% BSA/PBS for 2
379 h at room temperature. Sections were again washed four times in 1×PBS for 10 min each, before
380 incubation with Wheat Germ Agglutinin (WGA) (Alexa Fluor® 594 Conjugate; cat. no. W11262,
381 Thermo Fisher Scientific, Waltham, MA), diluted to 1:100 in 1×PBS (from a 1.25 mg/mL stock
382 solution), for 15 min at room temperature. Sections were washed again 4 times with 1×PBS for 3 min
383 each, blotted dry with a Kim-Wipe, and Flourosshield™ (cat. no. F6182; Sigma Aldrich, St Louis, MO)
384 added to each section before the coverslip was mounted. Stained muscle sections were air-dried for ~2
385 h and viewed with an Olympus BX51 microscope coupled with an Olympus DP72 camera for
386 fluorescence detection (Olympus, Shinjuku, Japan). Images were captured with a 10× objective and
387 analysed using Image Pro Premier software (version 9.1; Media Cybernetics, Rockville, MD).
388 Analysis was completed by an investigator blinded to all groups and time points. For each subject,
389 muscle fibre CSA was determined for both type I and type II muscle fibres. For the RT, HIT+RT and
390 MICT+RT groups, a total of 107 ± 61 , 112 ± 67 , and 84 ± 73 (mean \pm SD) type I fibres and 154 ± 72 ,
391 136 ± 80 , and 144 ± 76 (mean \pm SD) type II fibres were included for analysis, respectively.

392

393

394

395

396

397 **6. Statistical analyses**

398 The effect of training group on outcomes was evaluated via a two-way (time \times group) analysis of
399 variance with repeated-measures (RM-ANOVA) (SPSS, Version 21, IBM Corporation, New York,
400 NY). Western blot, qPCR and immunohistochemistry data were log-transformed before analysis to
401 reduce non-uniformity of error (Hopkins *et al.*, 2009). To quantify the magnitude of within- and
402 between-group differences for dependent variables, a magnitude-based approach to inferences using
403 the standardised difference (effect size, ES) was used (Hopkins *et al.*, 2009). The magnitude of effects
404 were defined according to thresholds suggested by Hopkins (Hopkins *et al.*, 2009), whereby $<0.2 =$
405 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
406 large effects. Lacking information on the smallest meaningful effect for changes in protein
407 phosphorylation and gene expression, the threshold for the smallest worthwhile effect was defined as
408 an ES of 0.4, rather than the conventional threshold of 0.2 (Fyfe *et al.*, 2016b). Magnitude-based
409 inferences about effects were made by qualifying the effects with probabilities reflecting the
410 uncertainty in the magnitude of the true effect (Batterham & Hopkins, 2005). Effects that were deemed
411 substantial in magnitude (and therefore meaningful) were those at least 75% ‘likely’ to exceed the
412 smallest worthwhile effect (according to the overlap between the uncertainty in the magnitude of the
413 true effect and the smallest worthwhile change (Batterham & Hopkins, 2005)). Exact *P* values were
414 also determined for each comparison, derived from paired (for within-group comparisons) or unpaired
415 (for between-group comparisons) *t*-tests, with a Bonferroni correction applied to correct for multiple
416 comparisons (SPSS, Version 21, IBM Corporation, New York, NY). A summary of all within- and
417 between-group comparisons for this study are presented in supplementary tables 1 and 2, respectively.
418 Physiological (blood lactate, blood glucose, heart rate) and psychological (rating of perceived exertion
419 [RPE]) responses to exercise are reported as mean values \pm SD, whereas protein phosphorylation and
420 gene expression data are reported as mean within- and between-condition percentage differences ± 90
421 % CL.

422 7. Results

423 *Training-induced changes in maximal strength and lean mass*

424 In brief, and as previously reported (Fyfe *et al.*, 2016a), 1-RM leg press strength was improved from
425 PRE-T to POST-T for RT (mean change \pm 90% confidence interval; $38.5 \pm 8.5\%$; effect size [ES] \pm 90%
426 confidence interval; 1.26 ± 0.24 ; $P < 0.001$), HIT+RT ($28.7 \pm 5.3\%$; ES, 1.17 ± 0.19 ; $P < 0.001$) and
427 MICT+RT ($27.5 \pm 4.6\%$, ES, 0.81 ± 0.12 ; $P < 0.001$); however, the magnitude of this change was greater
428 for RT vs. both HIT+RT ($7.4 \pm 8.7\%$; ES, 0.40 ± 0.40) and MICT+RT ($8.2 \pm 9.9\%$; ES, 0.60 ± 0.45).
429 There were no substantial between-group differences in 1-RM bench press strength gain. Lower-body
430 lean mass was similarly increased for RT ($4.1 \pm 2.0\%$; ES; 0.33 ± 0.16 ; $P = 0.023$) and MICT+RT (3.6
431 $\pm 2.4\%$; ES; 0.45 ± 0.30 ; $P = 0.052$); however, this increase was attenuated for HIT+RT ($1.8 \pm 1.6\%$; ES;
432 0.13 ± 0.12 ; $P = 0.069$).

433 434 *Physiological and psychological responses to the final training session*

435 Heart rate and rating of perceived exertion (RPE)

436 During the final training session, there was a higher average heart rate (mean difference range, 14 ± 12
437 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
438 (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.

439 440 *Venous blood lactate and glucose responses during the final training session*

441 During the final training session, venous blood lactate (Table 3) was higher for HIT compared with
442 MICT at all time points both during cycling (mean difference range, 0.8 ± 0.5 to 4.5 ± 1.1 mmol·L⁻¹;
443 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
444 ± 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)
445 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 $\pm 102\%$; 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 ($123 \pm 79\%$; ES, 0.45 ± 0.19 ;
469 $P = 0.002$), and +3 h ($241 \pm 315\%$; 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 ($52 \pm 46\%$; ES, 0.76 ± 0.89) and MICT+RT ($75 \pm 24\%$; ES,
472 1.31 ± 0.80), and lower for MICT+RT vs. HIT+RT ($-47 \pm 36\%$; 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 \times
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 $\pm 58\%$; ES, 0.82 ± 0.45 ; $P = 0.010$) and + 3 h ($125 \pm 72\%$; 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 ($32 \pm 23\%$; ES, 0.54 ± 0.46)
481 and MICT+RT ($37 \pm 27\%$; ES, 0.61 ± 0.55), and greater between POST-T and +3 h for RT
482 compared with both HIT+RT ($49 \pm 17\%$; ES, 0.92 ± 0.45) and MICT+RT ($64 \pm 12\%$; 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

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

489

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

491

492 *AMPK/mTORC1 signalling*

493 **p-AMPK^{Thr172}**. There was a main effect of time for AMPK^{Thr172} phosphorylation ($P = 0.033$).

494 The phosphorylation of AMPK^{Thr172} was unchanged at POST-T compared with PRE-T for all
495 training groups (Figure 4A). AMPK phosphorylation was, however, increased at +1 h
496 compared with POST-T for RT ($78 \pm 72\%$; ES, 0.34 ± 0.23 ; $P = 0.031$). The change in AMPK
497 phosphorylation between POST-T and +3 h was also greater for RT compared with MICT+RT
498 ($59 \pm 44\%$; ES, 0.79 ± 0.83) but not HIT+RT ($54 \pm 49\%$; ES, 0.69 ± 0.83).

499

500 **p-ACC^{Ser79}**. There was a time \times group interaction for ACC^{Ser79} phosphorylation ($P = 0.04$).

501 The phosphorylation of ACC^{Ser79} was unchanged at POST-T compared with PRE-T for all
502 training groups (Figure 4B). Compared with POST-T, ACC phosphorylation was reduced at
503 +1 h for both RT ($-36 \pm 22\%$; ES, -0.28 ± 0.20 ; $P = 0.026$) and MICT+RT ($46 \pm 20\%$; ES, -0.56
504 ± 0.33 ; $P = 0.016$), and reduced at +3 h compared with POST-T for RT ($45 \pm 20\%$; ES, -0.37
505 ± 0.22 ; $P = 0.012$). Compared with RT, the change in ACC phosphorylation was also greater
506 for HIT+RT between POST-T and both +1 h ($99 \pm 100\%$; ES, 0.65 ± 0.46) and +3 h (169
507 $\pm 168\%$; ES, 0.94 ± 0.56).

508

509 **p-mTOR^{Ser2448}**. There was a main effect of time for mTOR^{Ser2448} phosphorylation ($P = 0.001$).

510 The phosphorylation of mTOR^{Ser2448} was unchanged at POST-T compared with PRE-T for all
511 training groups (Figure 4C). Compared with POST-T, mTOR phosphorylation was increased

512 at +1 h for RT ($105 \pm 137\%$; ES, 0.46 ± 0.40 ; $P = 0.048$), but not for either HIT+RT ($30 \pm 71\%$;
513 ES, 0.32 ± 0.62 ; $P = 0.320$) or MICT+RT ($77 \pm 184\%$; ES, 0.37 ± 0.59 ; $P = 0.218$), and increased
514 at +3 h for compared with POST-T for HIT+RT ($70 \pm 45\%$; 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 ($95 \pm 47\%$; 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 ($78 \pm 77\%$;
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 ($47 \pm 50\%$; ES, 0.86 ± 1.13) and MICT+RT ($50 \pm 46\%$; 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: $700 \pm 678\%$; ES, 0.75 ± 0.28 ; $P < 0.001$; HIT+RT: $475 \pm 572\%$;
530 ES, 0.66 ± 0.33 ; $P = 0.005$; MICT+RT: $621 \pm 420\%$; ES, 1.49 ± 0.42 ; $P < 0.001$) and +3 h (RT:
531 $967 \pm 1047\%$; ES, 0.85 ± 0.31 ; $P < 0.001$; HIT+RT: $294 \pm 319\%$; ES, 0.51 ± 0.28 ; $P = 0.006$;
532 MICT+RT: $176 \pm 200\%$; 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 ($74 \pm 29\%$; ES, 0.72 ± 0.51) but not HIT+RT ($63 \pm 41\%$; ES, 0.57 ± 0.56).

535

536 **p-4E-BP1^{Thr56/47}**. There was a main effect of group for 4E-BP1^{Thr36/47} phosphorylation ($P <$
537 0.001; Figure 4F); however, there were no between-group differences in 4E-BP1^{Thr36/47}
538 phosphorylation at any time point.

539

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

541

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

543

544 *Ribosomal RNA (rRNA) responses*

545 **Total RNA content.** Total RNA content was used as an index of total translational capacity of
546 skeletal muscle, since ribosomal RNA comprises over 85% of the total RNA pool (Haddad *et*
547 *al.*, 2005). There was a time \times group interaction for changes in total RNA content ($P = 0.008$).
548 At PRE, total RNA content was higher for RT compared with both HIT+RT ($38 \pm 17\%$; ES, -
549 1.48 ± 0.84 ; $P = 0.005$; Table 5) and MICT+RT ($25 \pm 12\%$; ES, 1.47 ± 0.85 ; $P = 0.010$). Total
550 RNA content decreased between PRE-T and POST-T for RT ($-11 \pm 5\%$; ES, -0.17 ± 0.09 ; $P =$
551 0.025). Conversely, total RNA content was not substantially changed between PRE-T and
552 POST-T for both HIT+RT ($32 \pm 18\%$; ES, 0.30 ± 0.15 ; $P = 0.077$) and MICT+RT ($20 \pm 15\%$;
553 ES, 0.12 ± 0.08 ; $P = 0.083$). The PRE-T to POST-T change in total RNA content was, however,
554 greater for both HIT+RT ($48 \pm 39\%$; ES, 1.14 ± 0.76) and MICT+RT ($34 \pm 24\%$; ES, 1.24 ± 0.75)
555 compared with RT.

556

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

558

559 **45S pre-rRNA.** There was a main effect of time for changes in 45S pre-rRNA expression (P
560 < 0.001). Expression of 45S pre-rRNA was unchanged at POST-T compared with PRE-T for
561 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 ($58 \pm 76\%$; ES, 0.71 ± 0.71) and MICT+RT (75
563 $\pm 81\%$; 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 ($-51 \pm 16\%$; 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 ($125 \pm 109\%$; ES, 1.27 ± 0.73)
573 and MICT+RT ($120 \pm 111\%$; 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 \times 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 ($-36 \pm 15\%$; 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 ($112 \pm 116\%$; 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

587 **18S rRNA (span).** There were no substantial effects of training or any between-group
588 differences in changes in 18S rRNA (span) expression (Figure 7D), although a small increase
589 in 18S rRNA (span) expression was noted at +3 h compared with POST-T for MICT+RT (63
590 $\pm 48\%$; ES, 0.21 ± 0.12 ; $P = 0.029$).

591

592 **28S rRNA (mature).** Expression of 28S rRNA was reduced at POST-T compared with PRE-
593 T for RT ($-33 \pm 15\%$; ES, -0.49 ± 0.28 ; $P = 0.037$; Figure 7E); however, this effect was only
594 possibly substantial. The change in 28S rRNA expression between PRE-T and POST-T was
595 also greater for both HIT+RT ($73 \pm 56\%$; ES, 1.23 ± 0.71 ; $P = 0.007$) and MICT+RT ($63 \pm 55\%$;
596 ES, 1.10 ± 0.74 ; $P = 0.023$) compared with RT. There were no substantial changes in 28S rRNA
597 expression between POST-T and +3 h for either training group.

598

599 **28S rRNA (span).** There was a main effect of group for changes in 28S rRNA (span)
600 expression ($P < 0.001$). There were no substantial changes in 28S rRNA (span) expression
601 between PRE-T and POST-T for either training group (Figure 7F). However, the change in 28S
602 rRNA (span) expression between PRE-T and POST-T was greater for HIT+RT compared with
603 RT ($123 \pm 109\%$; ES, 0.81 ± 0.48).

604

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

606

607

608 *mRNA responses*

609 **TIF-1A mRNA.** There was a main effect of time for changes in TIF-1A mRNA expression (P
610 $= 0.008$). Expression of TIF-1A mRNA was unchanged at POST-T compared with PRE-T for
611 all training groups (Figure 8A). Compared with POST-T, TIF-1A expression was increased at

612 +3 h for both RT ($26 \pm 12\%$; ES, 0.53 ± 0.21 ; $P = 0.003$) and MICT+RT ($36 \pm 35\%$; 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 \times 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 ($-26 \pm 16\%$; 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 ($44 \pm 42\%$; ES, 0.57 ± 0.44 ; $P = 0.047$) and MICT+RT ($48 \pm 43\%$; 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 ($37 \pm 30\%$; ES, 0.87 ± 0.60) and POST-T -+3 h ($34 \pm 51\%$; 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 ($101 \pm 54\%$; 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 \times 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 ($206 \pm 163\%$; 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 ($168 \pm 176\%$; ES,
643 2.15 ± 1.34) and MICT+RT ($60 \pm 34\%$; ES, 1.85 ± 1.56).

644

645 **Atrogin-1 mRNA.** There were main effects of time ($P = 0.028$) and a time \times 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 ($-44 \pm 22\%$; ES, -0.91 ± 0.60 ; $P =$
649 0.018), but not substantially changed for either HIT+RT or 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 ($-89 \pm 83\%$; ES, -1.22 ± 0.82) and MICT+RT ($-86 \pm 89\%$; 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 ($28 \pm 17\%$;
655 ES, 0.49 ± 0.27 ; $P = 0.051$), but unchanged 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 ($158 \pm 65\%$; 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 ($141 \pm 73\%$; ES, 0.80 ± 0.27) and
659 MICT+RT ($47 \pm 31\%$; ES, 0.54 ± 0.47).

660

661 **PGC-1 α mRNA.** There were main effects of time ($P < 0.001$), group ($P < 0.001$), and a time
662 \times group interaction ($P < 0.001$), for changes in PGC-1 α mRNA expression (Figure 9D).
663 Compared with POST-T, PGC-1 α mRNA expression was increased at +3 h for both HIT+RT
664 (826 \pm 349%; ES, 4.58 \pm 0.76; $P < 0.001$) and MICT+RT (590 \pm 481%; ES, 1.97 \pm 0.66; $P =$
665 0.001), but unchanged for RT. The change in PGC-1 α mRNA expression between POST-T and
666 +3 h was greater for both HIT+RT (635 \pm 360%; ES, 4.80 \pm 1.14) and MICT+RT (447 \pm 379%;
667 ES, 2.75 \pm 1.05) compared with RT.

668

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

670

671 *Muscle fibre CSA responses*

672 Type I muscle fibre CSA (see Table 5) was increased at POST-T compared with PRE-T for
673 RT (15 \pm 13%; ES, 0.10 \pm 0.08; $P = 0.035$), but was not substantially changed for either HIT+RT
674 (-23 \pm 19%; ES, -0.09 \pm 0.08; $P = 0.135$) or MICT+RT (0.4 \pm 17%; ES, 0.00 \pm -0.14; $P = 0.989$).
675 The training-induced change in type I fibre CSA was also substantially greater for RT
676 compared with HIT+RT (34 \pm 22%; ES, 1.03 \pm 0.80), but not MICT+RT (15 \pm 54%; ES, 0.39
677 \pm 1.45).

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

683

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

685

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

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

742

743 In addition to mediating transient changes in translational efficiency, accumulating evidence
744 suggests mTORC1 also plays a critical role in regulating ribosome biogenesis (and therefore
745 translational capacity) in skeletal muscle by regulating all three classes of RNA polymerases
746 (RNA Pol-I to -III) (Iadevaia *et al.*, 2014). Inhibition of mTORC1 by rapamycin leads to the
747 inactivation of TIF-1A, which impairs the recruitment of RNA Pol-I-associated transcription-
748 initiation complexes mediating the transcription of 45S pre-rRNA genes (Mayer *et al.*, 2004).
749 The key mTORC1 substrate p70S6K1 also plays a role in mediating Pol-I activity via its
750 interaction with UBF, a transcription factor that interacts with the RNA Pol-I machinery via
751 SL-1 (Hannan *et al.*, 2003). In agreement with mTORC1 signalling responses, the
752 phosphorylation of upstream regulators of RNA Pol-I-mediated rDNA transcription, including
753 UBF and TIF-1A, was increased more by RE alone than when combined with either HIT or
754 MICT. Previous work has demonstrated single sessions of RE to induce robust increases in
755 TIF-1A Ser⁶⁴⁹ phosphorylation and UBF protein content in human skeletal muscle at 1 h post-
756 exercise, both in untrained and trained states (Figueiredo *et al.*, 2015). Moreover, whereas a
757 single session of RE did not impact upon UBF Ser³⁸⁸ phosphorylation, this response was
758 elevated in the basal state post-training (Figueiredo *et al.*, 2015). The present data add to the
759 growing body of evidence that RE is a potent stimulus for increasing the phosphorylation of
760 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-1A 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-rRNA, 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 polycistronic 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

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

862

863 Despite the changes in skeletal muscle RNA content, RT was sufficient to increase type I
864 muscle fibre CSA. In agreement with previous research (Kraemer *et al.*, 1995; Bell *et al.*,
865 2000), the training-induced increase in type I muscle fibre CSA was attenuated with concurrent
866 exercise, albeit only when incorporating HIT, compared with RT performed alone. Despite
867 these between-group differences in fibre-type specific hypertrophy, we could find no evidence
868 that the training-induced changes in lean mass or muscle fibre CSA were correlated with
869 changes in total RNA content of skeletal muscle (data not shown). The apparent disconnect
870 between training-induced changes in total RNA content and markers of muscle hypertrophy,
871 both at the whole-body and muscle-fibre levels, suggests further investigation is required into
872 relationship between changes in translational capacity and RT-induced hypertrophy in human
873 skeletal muscle.

874

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

885 combination with MICT. While the role of Atrogin-1 in mediating protein degradation is less
886 clear compared with MuRF-1 (Krawiec *et al.*, 2005), we nevertheless observed a reduction in
887 Atrogin-1 expression at +3 h for RE, but not for either concurrent exercise group. These data
888 are consistent with previous reports of reduced Atrogin-1 expression 3 h after RE performed
889 in both untrained and trained states (Fernandez-Gonzalo *et al.*, 2013), but contrast others
890 showing reduced Atrogin-1 expression 3 h after RE only when preceded 6 h earlier by MICT
891 (40 min cycling at 70% of peak power output) (Lundberg *et al.*, 2012). Taken together, these
892 data suggest concurrent exercise incorporating HIT may exacerbate post-exercise rates of
893 protein degradation by increasing MuRF-1 mRNA expression, while both concurrent exercise
894 protocols prevented the acute reduction in Atrogin-1 expression induced by RE alone. These
895 data should, however, be considered with recent evidence suggesting increased rates of protein
896 degradation may be necessary to promote skeletal muscle remodelling and be permissive,
897 rather than inhibitory, for training adaptations in skeletal muscle (Vainshtein & Hood, 2015).

898

899 **9. Conclusions**

900 This is the first study to simultaneously investigate markers of ribosome biogenesis and
901 mTORC1 signalling in human skeletal muscle following concurrent training compared with
902 RT performed alone. Contrary to our hypotheses, and recent work in humans (Nader *et al.*,
903 2014; Figueiredo *et al.*, 2015), we noted little evidence of ribosome biogenesis in skeletal
904 muscle following eight weeks of RT. Rather, increases in markers of ribosome biogenesis,
905 albeit small in magnitude, tended to be greater following concurrent exercise and were
906 independent of the endurance training intensity employed. This occurred despite a single
907 session of RE being a more potent stimulus for both mTORC1 signalling and phosphorylation
908 of regulators of RNA Pol-1-mediated rDNA transcription (i.e., TIF-1A and UBF) when
909 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

924 **10. References**

- 925 Adams GR, Caiozzo VJ, Haddad F & Baldwin KM. (2002). Cellular and molecular responses
926 to increased skeletal muscle loading after irradiation. *Am J Physiol Cell Physiol* **283**,
927 C1182-1195.
- 928
929 Adams GR, Haddad F & Baldwin KM. (1999). Time course of changes in markers of
930 myogenesis in overloaded rat skeletal muscles. *J Appl Physiol (1985)* **87**, 1705-1712.
- 931
932 Apro W, Moberg M, Hamilton DL, Ekblom B, van Hall G, Holmberg HC & Blomstrand E.
933 (2015). Resistance exercise-induced S6K1 kinase activity is not inhibited in human
934 skeletal muscle despite prior activation of AMPK by high-intensity interval cycling.
935 *Am J Physiol Endocrinol Metab* **308**, E470-481.
- 936
937 Apro W, Wang L, Ponten M, Blomstrand E & Sahlin K. (2013). Resistance exercise induced
938 mTORC1 signalling is not impaired by subsequent endurance exercise in human
939 skeletal muscle. *Am J Physiol Endocrinol Metab* **305**, E22-32.
- 940
941 Atherton PJ, Babraj J, Smith K, Singh J, Rennie MJ & Wackerhage H. (2005). Selective
942 activation of AMPK-PGC-1 α or PKB-TSC2-mTOR signaling can explain specific
943 adaptive responses to endurance or resistance training-like electrical muscle
944 stimulation. *FASEB J* **19**, 786-788.
- 945
946 Atherton PJ & Smith K. (2012). Muscle protein synthesis in response to nutrition and
947 exercise. *J Physiol* **590**, 1049-1057.
- 948
949 Batterham AM & Hopkins WG. (2005). Making Meaningful Inferences About Magnitudes.
950 In *Sportscience*.
- 951
952 Bell GJ, Syrotuik D, Martin TP, Burnham R & Quinney HA. (2000). Effect of concurrent
953 strength and endurance training on skeletal muscle properties and hormone
954 concentrations in humans. *Eur J Appl Physiol* **81**, 418-427.
- 955
956 Bergstrom J. (1962). Muscle electrolytes in man. *Scand J Clin Lab Invest* **68**, 1-110.
- 957
958 Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro
959 FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN,
960 Yancopoulos GD & Glass DJ. (2001a). Identification of ubiquitin ligases required for
961 skeletal muscle atrophy. *Science* **294**, 1704-1708.
- 962
963 Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E,
964 Scrimgeour A, Lawrence JC, Glass DJ & Yancopoulos GD. (2001b). Akt/mTOR

- 965 pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle
966 atrophy in vivo. *Nat Cell Biol* **3**, 1014-1019.
- 967
- 968 Carrithers JA, Carroll CC, Coker RH, Sullivan DH & Trappe TA. (2007). Concurrent
969 exercise and muscle protein synthesis: implications for exercise countermeasures in
970 space. *Aviat Space Environ Med* **78**, 457-462.
- 971
- 972 Chaillou T, Kirby TJ & McCarthy JJ. (2014). Ribosome biogenesis: emerging evidence for a
973 central role in the regulation of skeletal muscle mass. *Journal of cellular physiology*
974 **229**, 1584-1594.
- 975
- 976 Chaillou T, Koulmann N, Simler N, Meunier A, Serrurier B, Chapot R, Peinnequin A,
977 Beaudry M & Bigard X. (2012). Hypoxia transiently affects skeletal muscle
978 hypertrophy in a functional overload model. *Am J Physiol Regul Integr Comp Physiol*
979 **302**, R643-654.
- 980
- 981 Chaillou T, Lee JD, England JH, Esser KA & McCarthy JJ. (2013). Time course of gene
982 expression during mouse skeletal muscle hypertrophy. *J Appl Physiol (1985)* **115**,
983 1065-1074.
- 984
- 985 Coffey VG, Jemiolo B, Edge J, Garnham AP, Trappe SW & Hawley JA. (2009a). Effect of
986 consecutive repeated sprint and resistance exercise bouts on acute adaptive responses
987 in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* **297**, R1441-1451.
- 988
- 989 Coffey VG, Pilegaard H, Garnham AP, O'Brien BJ & Hawley JA. (2009b). Consecutive
990 bouts of diverse contractile activity alter acute responses in human skeletal muscle. *J*
991 *Appl Physiol* **106**, 1187-1197.
- 992
- 993 Donges CE, Burd NA, Duffield R, Smith GC, West DW, Short MJ, Mackenzie RW, Plank
994 LD, Shepherd PR, Phillips SM & Edge JA. (2012). Concurrent resistance and aerobic
995 exercise stimulates both myofibrillar and mitochondrial protein synthesis in sedentary
996 middle-aged men. *J Appl Physiol* **112**, 1992-2001.
- 997
- 998 Drummond MJ, Fry CS, Glynn EL, Dreyer HC, Dhanani S, Timmerman KL, Volpi E &
999 Rasmussen BB. (2009). Rapamycin administration in humans blocks the contraction-
1000 induced increase in skeletal muscle protein synthesis. *J Physiol* **587**, 1535-1546.
- 1001
- 1002 Evans WJ, Phinney SD & Young VR. (1982). Suction applied to a muscle biopsy maximizes
1003 sample size. *Med Sci Sports Exerc* **14**, 101-102.
- 1004
- 1005 Fernandez-Gonzalo R, Lundberg TR & Tesch PA. (2013). Acute molecular responses in
1006 untrained and trained muscle subjected to aerobic and resistance exercise training
1007 versus resistance training alone. *Acta Physiol (Oxf)* **209**, 283-294.

- 1008
1009 Figueiredo VC, Caldow MK, Massie V, Markworth JF, Cameron-Smith D & Blazevich AJ.
1010 (2015). Ribosome biogenesis adaptation in resistance training-induced human skeletal
1011 muscle hypertrophy. *Am J Physiol Endocrinol Metab* **309**, E72-83.
- 1012
1013 Figueiredo VC, Roberts LA, Markworth JF, Barnett MP, Coombes JS, Raastad T, Peake JM
1014 & Cameron-Smith D. (2016). Impact of resistance exercise on ribosome biogenesis is
1015 acutely regulated by post-exercise recovery strategies. *Physiological reports* **4**.
- 1016
1017 Fyfe JJ, Bartlett JD, Hanson ED, Stepto NK & Bishop DJ. (2016a). Endurance training
1018 intensity does not mediate interference to maximal lower-body strength gain during
1019 short-term concurrent training. *Frontiers in physiology* **Nov 3;7**.
- 1020
1021 Fyfe JJ, Bishop DJ & Stepto NK. (2014). Interference between Concurrent Resistance and
1022 Endurance Exercise: Molecular Bases and the Role of Individual Training Variables.
1023 *Sports Med* **44**, 743-762.
- 1024
1025 Fyfe JJ, Bishop DJ, Zacharewicz E, Russell AP & Stepto NK. (2016b). Concurrent exercise
1026 incorporating high-intensity interval or continuous training modulates mTORC1
1027 signalling and microRNA expression in human skeletal muscle. *Am J Physiol Regul*
1028 *Integr Comp Physiol*, ajpregu 00479 02015.
- 1029
1030 Goodman CA, Frey JW, Mabrey DM, Jacobs BL, Lincoln HC, You JS & Hornberger TA.
1031 (2011a). The role of skeletal muscle mTOR in the regulation of mechanical load-
1032 induced growth. *J Physiol* **589**, 5485-5501.
- 1033
1034 Goodman CA, Mabrey DM, Frey JW, Miu MH, Schmidt EK, Pierre P & Hornberger TA.
1035 (2011b). Novel insights into the regulation of skeletal muscle protein synthesis as
1036 revealed by a new nonradioactive in vivo technique. *FASEB J* **25**, 1028-1039.
- 1037
1038 Haddad F, Baldwin KM & Tesch PA. (2005). Pretranslational markers of contractile protein
1039 expression in human skeletal muscle: effect of limb unloading plus resistance
1040 exercise. *J Appl Physiol (1985)* **98**, 46-52.
- 1041
1042 Hannan KM, Brandenburger Y, Jenkins A, Sharkey K, Cavanaugh A, Rothblum L, Moss T,
1043 Poortinga G, McArthur GA, Pearson RB & Hannan RD. (2003). mTOR-dependent
1044 regulation of ribosomal gene transcription requires S6K1 and is mediated by
1045 phosphorylation of the carboxy-terminal activation domain of the nucleolar
1046 transcription factor UBF. *Mol Cell Biol* **23**, 8862-8877.
- 1047
1048 Hickson RC. (1980). Interference of strength development by simultaneously training for
1049 strength and endurance. *Eur J Appl Physiol Occup Physiol* **45**, 255-263.
- 1050

- 1051 Hopkins WG, Marshall SW, Batterham AM & Hanin J. (2009). Progressive statistics for
1052 studies in sports medicine and exercise science. *Med Sci Sports Exerc* **41**, 3-13.
- 1053
- 1054 Iadevaia V, Liu R & Proud CG. (2014). mTORC1 signaling controls multiple steps in
1055 ribosome biogenesis. *Seminars in cell & developmental biology* **36**, 113-120.
- 1056
- 1057 Kadi F, Schjerling P, Andersen LL, Charifi N, Madsen JL, Christensen LR & Andersen JL.
1058 (2004). The effects of heavy resistance training and detraining on satellite cells in
1059 human skeletal muscles. *J Physiol* **558**, 1005-1012.
- 1060
- 1061 Kantidakis T, Ramsbottom BA, Birch JL, Dowding SN & White RJ. (2010). mTOR
1062 associates with TFIIC, is found at tRNA and 5S rRNA genes, and targets their
1063 repressor Maf1. *Proc Natl Acad Sci U S A* **107**, 11823-11828.
- 1064
- 1065 Kimball SR. (2006). Interaction between the AMP-activated protein kinase and mTOR
1066 signaling pathways. *Med Sci Sports Exerc* **38**, 1958-1964.
- 1067
- 1068 Kirby TJ, Lee JD, England JH, Chaillou T, Esser KA & McCarthy JJ. (2015). Blunted
1069 hypertrophic response in aged skeletal muscle is associated with decreased ribosome
1070 biogenesis. *J Appl Physiol (1985)* **Aug 15;119**, 321-327.
- 1071
- 1072 Kraemer WJ, Patton JF, Gordon SE, Harman EA, Deschenes MR, Reynolds K, Newton RU,
1073 Triplett NT & Dziados JE. (1995). Compatibility of high-intensity strength and
1074 endurance training on hormonal and skeletal muscle adaptations. *J Appl Physiol* **78**,
1075 976-989.
- 1076
- 1077 Krawiec BJ, Frost RA, Vary TC, Jefferson LS & Lang CH. (2005). Hindlimb casting
1078 decreases muscle mass in part by proteasome-dependent proteolysis but independent
1079 of protein synthesis. *Am J Physiol Endocrinol Metab* **289**, E969-980.
- 1080
- 1081 Leveritt M, Abernethy PJ, Barry BK & Logan PA. (1999). Concurrent strength and
1082 endurance training. A review. *Sports Med* **28**, 413-427.
- 1083
- 1084 Livak KJ & Schmittgen TD. (2001). Analysis of relative gene expression data using real-time
1085 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408.
- 1086
- 1087 Lundberg TR, Fernandez-Gonzalo R, Gustafsson T & Tesch PA. (2012). Aerobic exercise
1088 alters skeletal muscle molecular responses to resistance exercise. *Med Sci Sports
1089 Exerc* **44**, 1680-1688.
- 1090

- 1091 Lundberg TR, Fernandez-Gonzalo R & Tesch PA. (2014). Exercise-induced AMPK
1092 activation does not interfere with muscle hypertrophy in response to resistance
1093 training in men. *J Appl Physiol (1985)* **116**, 611-620.
- 1094
1095 Mane VP, Heuer MA, Hillyer P, Navarro MB & Rabin RL. (2008). Systematic method for
1096 determining an ideal housekeeping gene for real-time PCR analysis. *J Biomol Tech*
1097 **19**, 342-347.
- 1098
1099 Mayer C, Zhao J, Yuan X & Grummt I. (2004). mTOR-dependent activation of the
1100 transcription factor TIF-IA links rRNA synthesis to nutrient availability. *Genes Dev*
1101 **18**, 423-434.
- 1102
1103 McConell GK, Lee-Young RS, Chen ZP, Stepto NK, Huynh NN, Stephens TJ, Canny BJ &
1104 Kemp BE. (2005). Short-term exercise training in humans reduces AMPK signalling
1105 during prolonged exercise independent of muscle glycogen. *J Physiol* **568**, 665-676.
- 1106
1107 McGee SL & Hargreaves M. (2010). AMPK-mediated regulation of transcription in skeletal
1108 muscle. *Clin Sci (Lond)* **118**, 507-518.
- 1109
1110 Miyazaki M, McCarthy JJ, Fedele MJ & Esser KA. (2011). Early activation of mTORC1
1111 signalling in response to mechanical overload is independent of phosphoinositide 3-
1112 kinase/Akt signalling. *J Physiol* **589**, 1831-1846.
- 1113
1114 Moss T & Stefanovsky VY. (1995). Promotion and regulation of ribosomal transcription in
1115 eukaryotes by RNA polymerase I. *Progress in nucleic acid research and molecular*
1116 *biology* **50**, 25-66.
- 1117
1118 Nader GA, von Walden F, Liu C, Lindvall J, Gutmann L, Pistilli EE & Gordon PM. (2014).
1119 Resistance exercise training modulates acute gene expression during human skeletal
1120 muscle hypertrophy. *J Appl Physiol (1985)* **116**, 693-702.
- 1121
1122 Nakada S, Ogasawara R, Kawada S, Maekawa T & Ishii N. (2016). Correlation between
1123 Ribosome Biogenesis and the Magnitude of Hypertrophy in Overloaded Skeletal
1124 Muscle. *PLoS One* **11**.
- 1125
1126 Pugh JK, Faulkner SH, Jackson AP, King JA & Nimmo MA. (2015). Acute molecular
1127 responses to concurrent resistance and high-intensity interval exercise in untrained
1128 skeletal muscle. *Physiological reports* **3**.
- 1129
1130 Stec MJ, Mayhew DL & Bamman MM. (2015). The effects of age and resistance loading on
1131 skeletal muscle ribosome biogenesis. *J Appl Physiol (1985)* **119**, 851-857.
- 1132

- 1133 Thomson DM, Fick CA & Gordon SE. (2008). AMPK activation attenuates S6K1, 4E-BP1,
1134 and eEF2 signaling responses to high-frequency electrically stimulated skeletal
1135 muscle contractions. *J Appl Physiol* **104**, 625-632.
- 1136
1137 Thomson E, Ferreira-Cerca S & Hurt E. (2013). Eukaryotic ribosome biogenesis at a glance.
1138 *J Cell Sci* **126**, 4815-4821.
- 1139
1140 Vainshtein A & Hood DA. (2015). The regulation of autophagy during exercise in skeletal
1141 muscle. *J Appl Physiol (1985)*, jap 00550 02015.
- 1142
1143 Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A & Speleman F.
1144 (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric
1145 averaging of multiple internal control genes. *Genome Biol* **3**, RESEARCH0034.
- 1146
1147 Vissing K, McGee SL, Farup J, Kjolhede T, Vendelbo MH & Jessen N. (2011).
1148 Differentiated mTOR but not AMPK signaling after strength vs endurance exercise in
1149 training-accustomed individuals. *Scand J Med Sci Sports* **23**, 355-366.
- 1150
1151 Voit R & Grummt I. (2001). Phosphorylation of UBF at serine 388 is required for interaction
1152 with RNA polymerase I and activation of rDNA transcription. *Proc Natl Acad Sci U S*
1153 *A* **98**, 13631-13636.
- 1154
1155 Voit R, Hoffmann M & Grummt I. (1999). Phosphorylation by G1-specific cdk-cyclin
1156 complexes activates the nucleolar transcription factor UBF. *EMBO J* **18**, 1891-1899.
- 1157
1158 von Walden F, Casagrande V, Ostlund Farrants AK & Nader GA. (2012). Mechanical
1159 loading induces the expression of a Pol I regulon at the onset of skeletal muscle
1160 hypertrophy. *Am J Physiol Cell Physiol* **302**, C1523-1530.
- 1161
1162 Wilkinson SB, Phillips SM, Atherton PJ, Patel R, Yarasheski KE, Tarnopolsky MA &
1163 Rennie MJ. (2008). Differential effects of resistance and endurance exercise in the fed
1164 state on signalling molecule phosphorylation and protein synthesis in human muscle.
1165 *J Physiol* **586**, 3701-3717.
- 1166
1167 Wilson JM, Marin PJ, Rhea MR, Wilson SM, Loenneke JP & Anderson JC. (2012).
1168 Concurrent training: a meta-analysis examining interference of aerobic and resistance
1169 exercises. *J Strength Cond Res* **26**, 2293-2307.
- 1170
1171
1172

1173 **11. Additional information section**

1174 *Competing interests*

1175 The authors declare no conflicts of interest relevant to the contents of this manuscript.

1176

1177 *Funding*

1178 This study was supported in part by a grant from the Gatorade Sports Science Institute (GSSI)

1179 awarded to J.J.F.

1180

1181 *Author contributions*

1182 • Study design was performed by J.J.F., J.D.B., E.D.H., D.J.B. and N.K.S. Data
1183 collection was performed by J.J.F, M.J.A and A.P.G. Analysis and interpretation of data
1184 was performed by J.J.F., J.D.B., E.D.H., D.J.B. and N.K.S. The manuscript was written
1185 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
1186 the manuscript. All authors approved the final version of the manuscript.

1187 • All data collection and data analysis for this study was conducted and performed in the
1188 exercise physiology and biochemistry laboratories at Victoria University, Footscray
1189 Park campus, Melbourne Australia.

1190 • All authors approved the final version of the manuscript and agree to be accountable
1191 for all aspects of the work in ensuring that questions related to the accuracy or integrity
1192 of any part of the work are appropriately investigated and resolved. All persons
1193 designated as authors qualify for authorship, and all those who qualify for authorship
1194 are listed.

1195

1196

1197

1198 *Acknowledgements*

1199 We gratefully acknowledge the efforts of the participants, without whom this study would not
1200 have been possible. We also acknowledge Dr Chris Shaw (Deakin University) for technical
1201 assistance with the immunofluorescence analysis.

1202 **12. Authors' translational perspective**

1203 Optimising adaptations to divergent exercise modes (i.e., resistance and endurance training) is
1204 important for maximising the associated health and exercise performance benefits. Concurrent
1205 training (i.e., combined resistance and endurance training) can result in compromised
1206 development of strength and muscle mass compared with resistance training performed alone.
1207 Despite this, the molecular mechanisms that underpin this altered adaptation to concurrent
1208 training in skeletal muscle are unclear. Recent evidence suggests ribosome biogenesis plays an
1209 important role in skeletal muscle hypertrophy; however, whether altered ribosome biogenesis
1210 occurs in human skeletal muscle with concurrent training is unclear. The present study has
1211 shown that ribosome biogenesis adaptation is not compromised following short-term
1212 concurrent training, despite attenuated signalling responses in skeletal muscle related to both
1213 ribosome biogenesis and translational efficiency (i.e., mTORC1 signalling) following a single
1214 session of concurrent exercise performed in a training-accustomed state. Importantly,
1215 compromised mTORC1 signalling does not appear to be evident when exercise is performed
1216 in untrained individuals, or in those not accustomed to the exercise protocol. The results of this
1217 study suggest that attenuated signalling responses related to changes in translational efficiency
1218 and capacity in skeletal muscle following single bouts of concurrent exercise, performed after
1219 a period of training, do not appear to be related to basal adaptive responses to short-term
1220 concurrent training. However, it is possible that these attenuated early post-exercise responses
1221 seen following concurrent exercise may underpin blunted adaptation to longer-term concurrent
1222 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'-CAGCCTCTGCATGATGTTTCAGT-3'	NM_058229.3
Fox-O1	5'-TTGTTACATAGTCAGCTTG-3'	5'-TCACTTTCCTGCCCAACCAG-3'	NM_002015.3
PGC-1 α	5'-GGCAGAAGGCAATTGAAGAG-3'	5'-TCAAAAACGGTCCCTCAGTTC-3'	NM_013261.3
UBF	5'-CCTGGGGAAGCAGTGGTCTC-3	5'-CCCTCCTCACTGATGTTTCAGC-3	XM_006722059.2
TIF-1A	5'-GTTTCGGTTTGGTGGAACTGTG-3	5'-TCTGGTCATCCTTTATGTCTGG-3	XM_005255377.3
Cyclin D1	5'-GCTGCGAAGTGGAACCATC-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
Cyclophilin	5'-GTCAACCCACCGTGTTC-3'	5'-TTTCTGCTGTCTTTGGGACCTTG-3'	XM_011508410.1
GAPDH	5'-AAAGCCTGCCGGTACTAAC-3'	5'-CGCCCAATACGACCAAATCAGA-3'	NM_001256799.2
β 2M	5'-TGCTGTCTCCATGTTTGATGTATCT-3'	5'-TCTCTGCTCCCCACCTCTAAGT-3'	NM_004048.2

MuRF-1, muscle RING-finger 1; Fox-O1, forkhead box-O1; PGC-1 α , 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; β 2M, 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·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·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·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·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·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

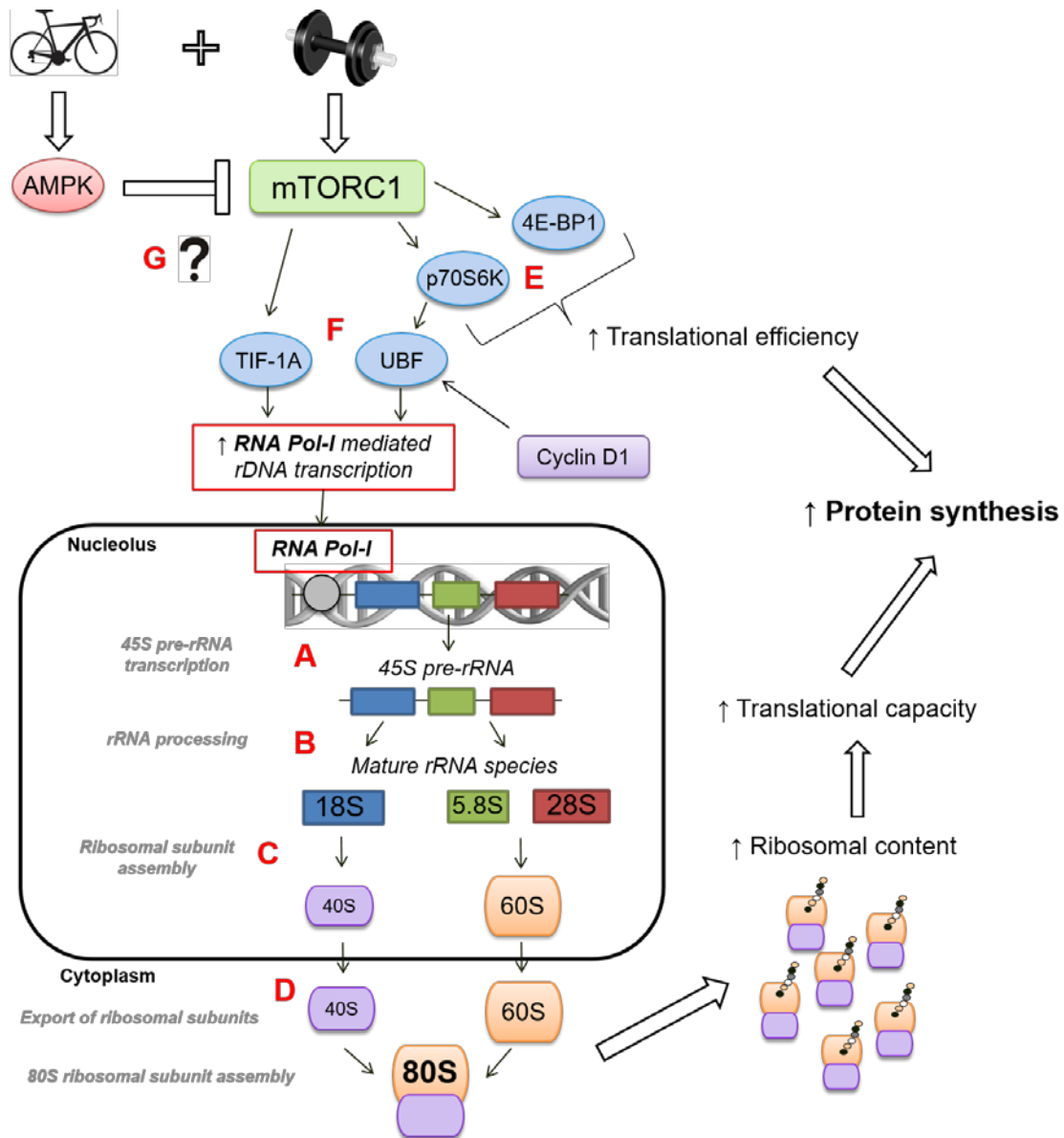


Figure 1. Overview of the role of mTORC1 signalling in promoting ribosome biogenesis following a single session of resistance exercise, and the potential effect of incorporating endurance training (i.e., performing concurrent training). Adapted from (Chaillou *et al.*, 2014). Ribosome biogenesis involves 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**).

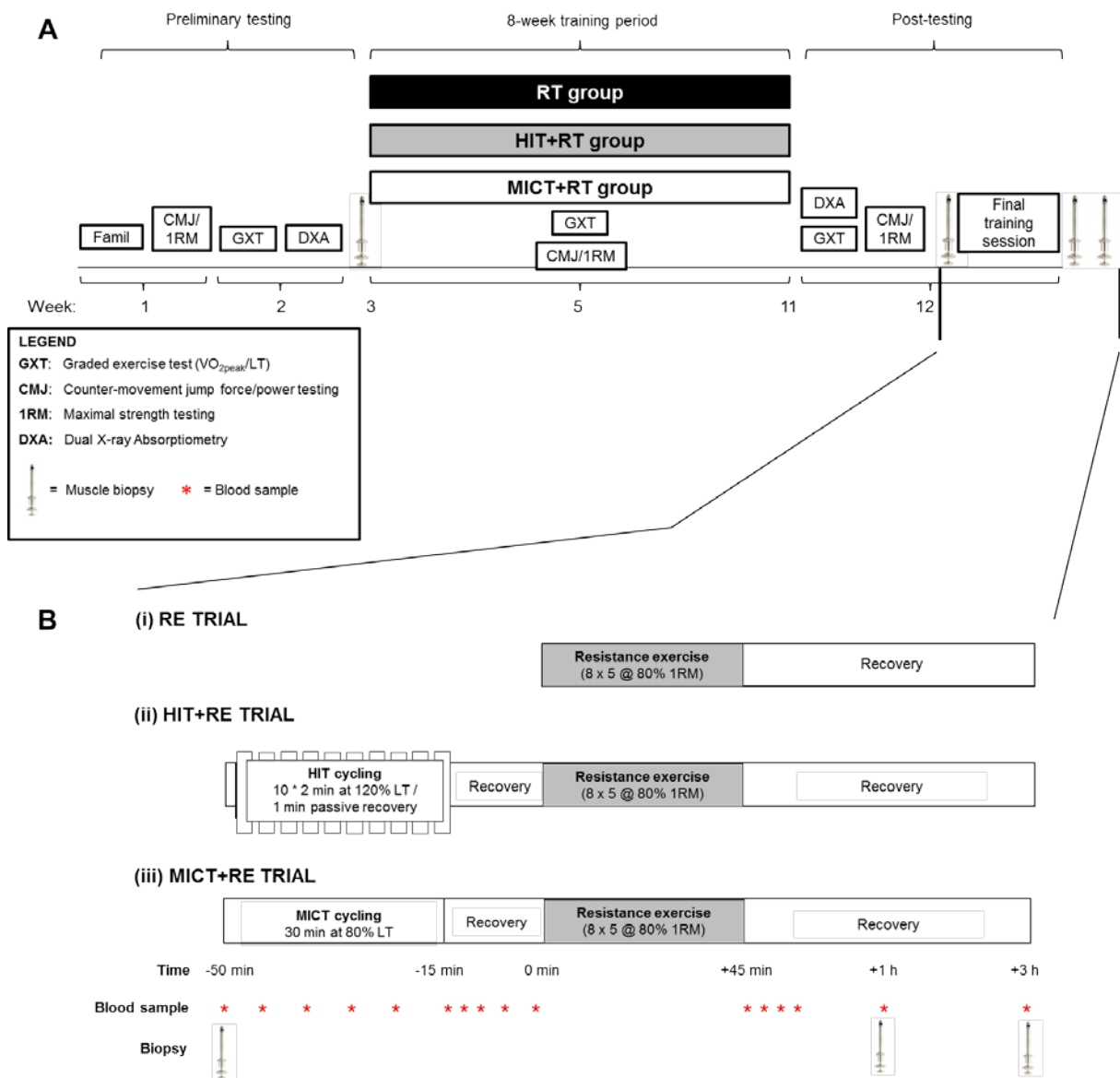


Figure 2. Study overview (A) and timelines for the final training session (B). Participants first completed 8 weeks of either resistance training (RT) alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT). For the final training 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.

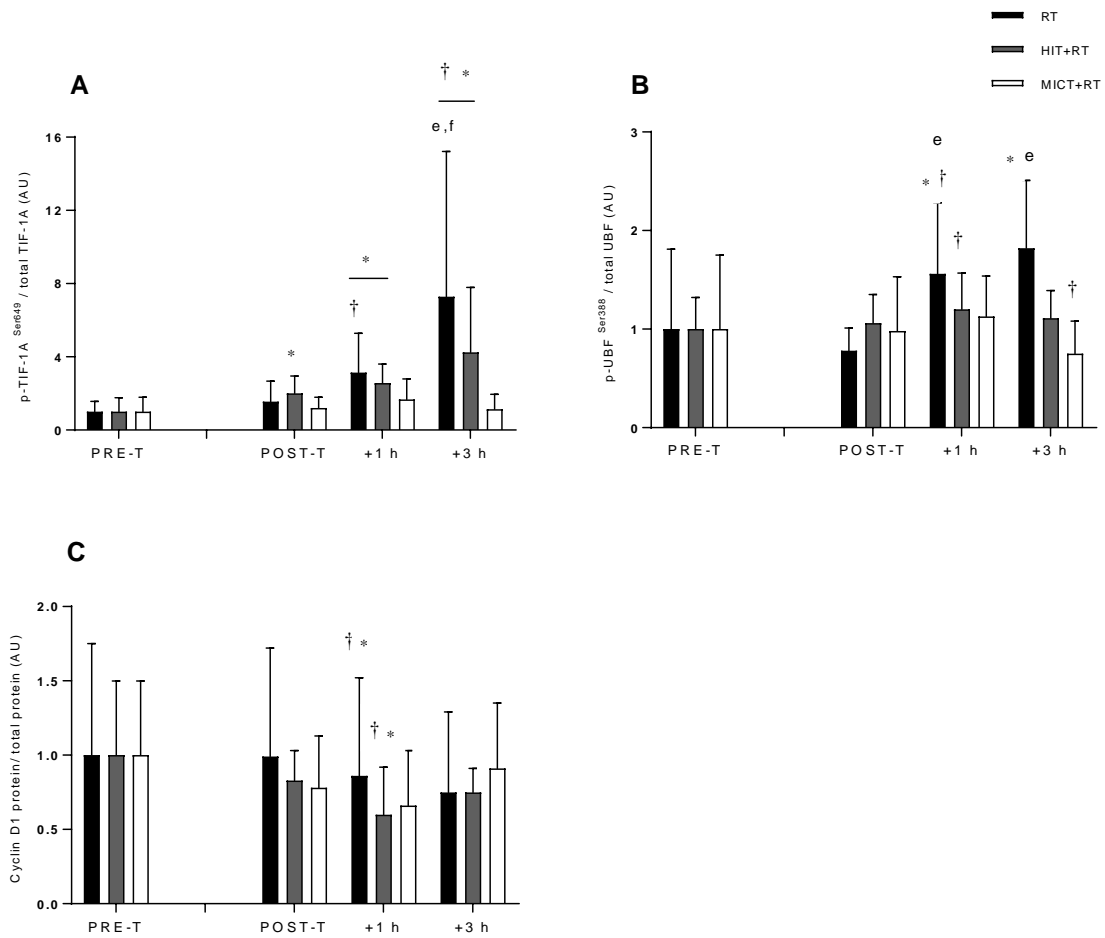


Figure 3. Phosphorylation of TIF-1A^{Ser649} (A), UBF^{Ser388} (B), and total protein content of cyclin D1 (C) before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means \pm 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.

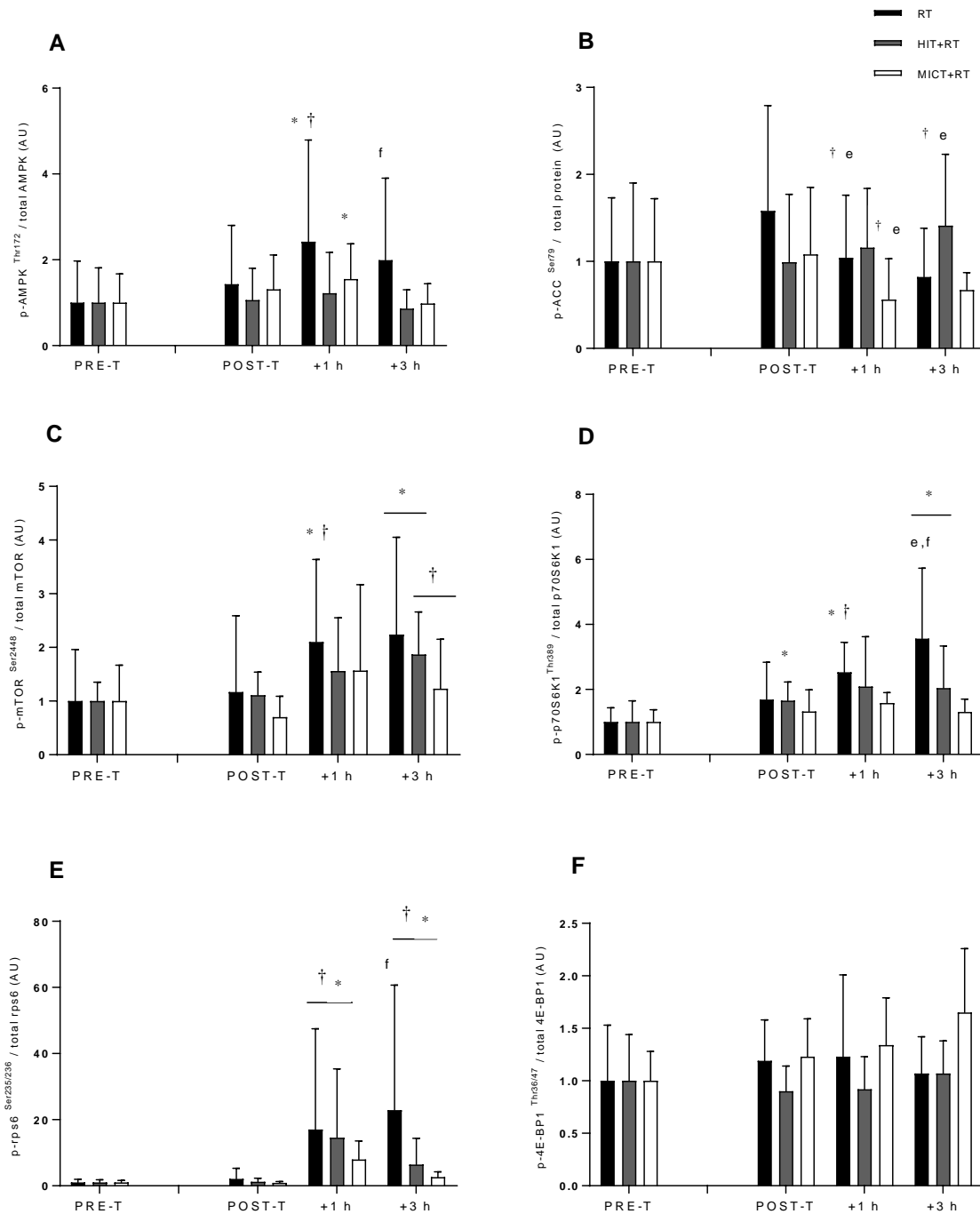


Figure 4. Phosphorylation of AMPK^{Thr172} (A), ACC^{Ser79} (B), mTOR^{Ser2448} (C), p70S6K^{Thr389} (D), rps6^{Ser235/236} (E) and 4E-BP1^{Thr36/47} (F) before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means \pm 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.

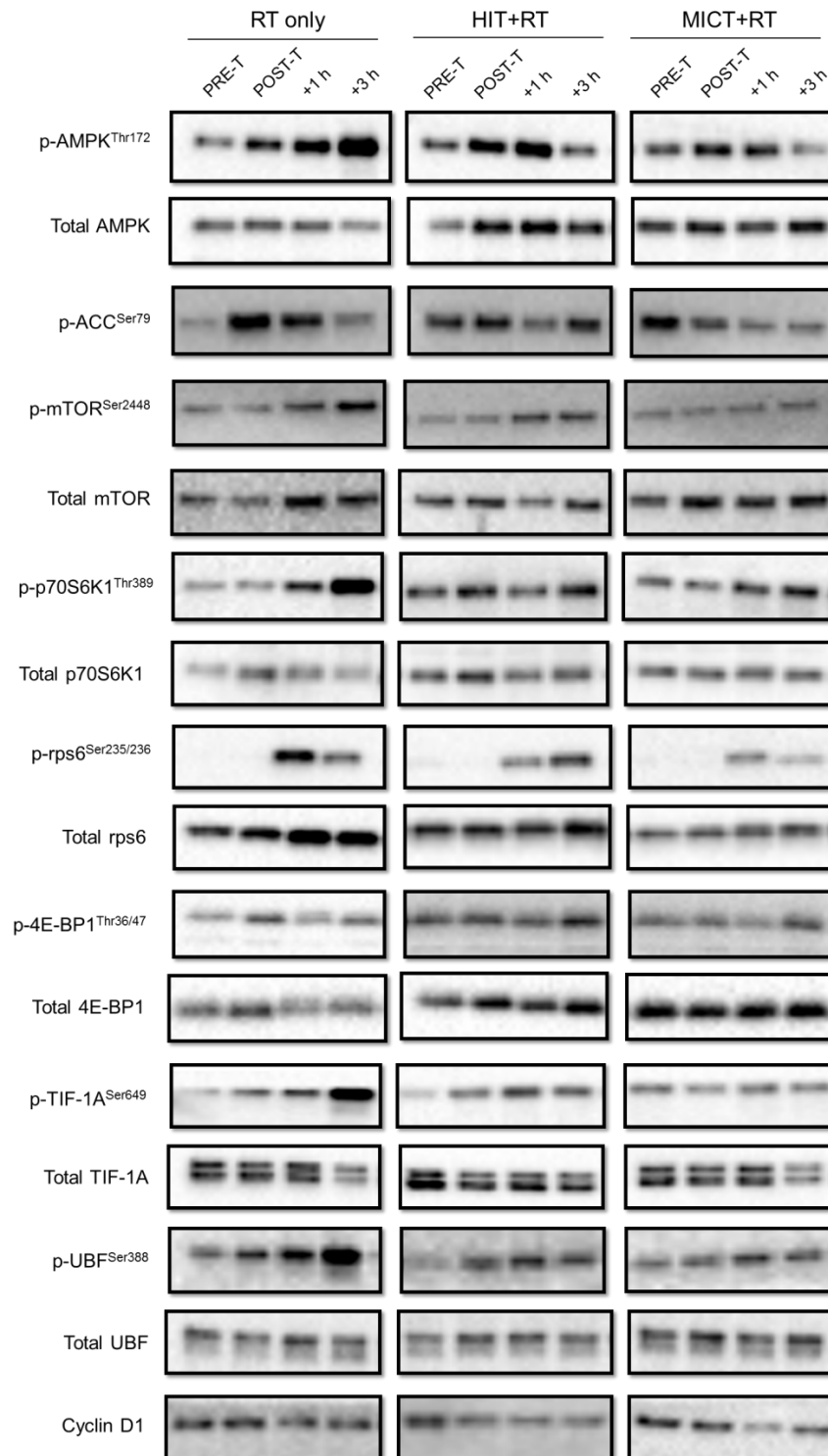


Figure 5. Representative western blots for the phosphorylation (p-) and total protein content of signalling proteins before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT),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

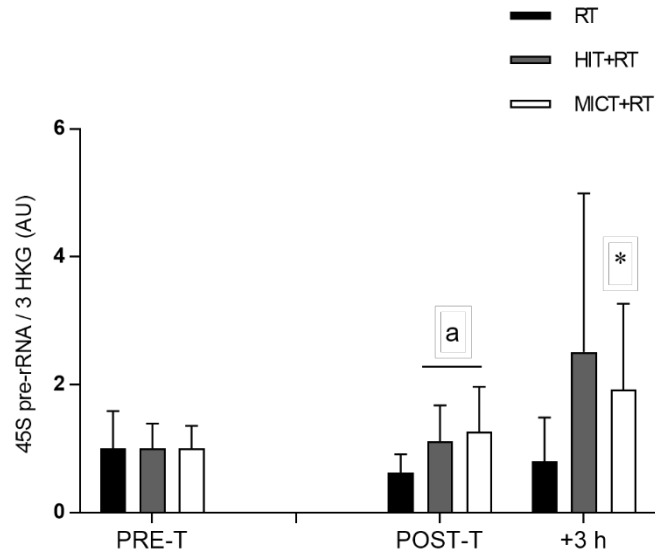


Figure 6. Expression of 45S pre-rRNA relative to the geometric mean of cyclophilin, β 2M and TBP expression before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means \pm 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.

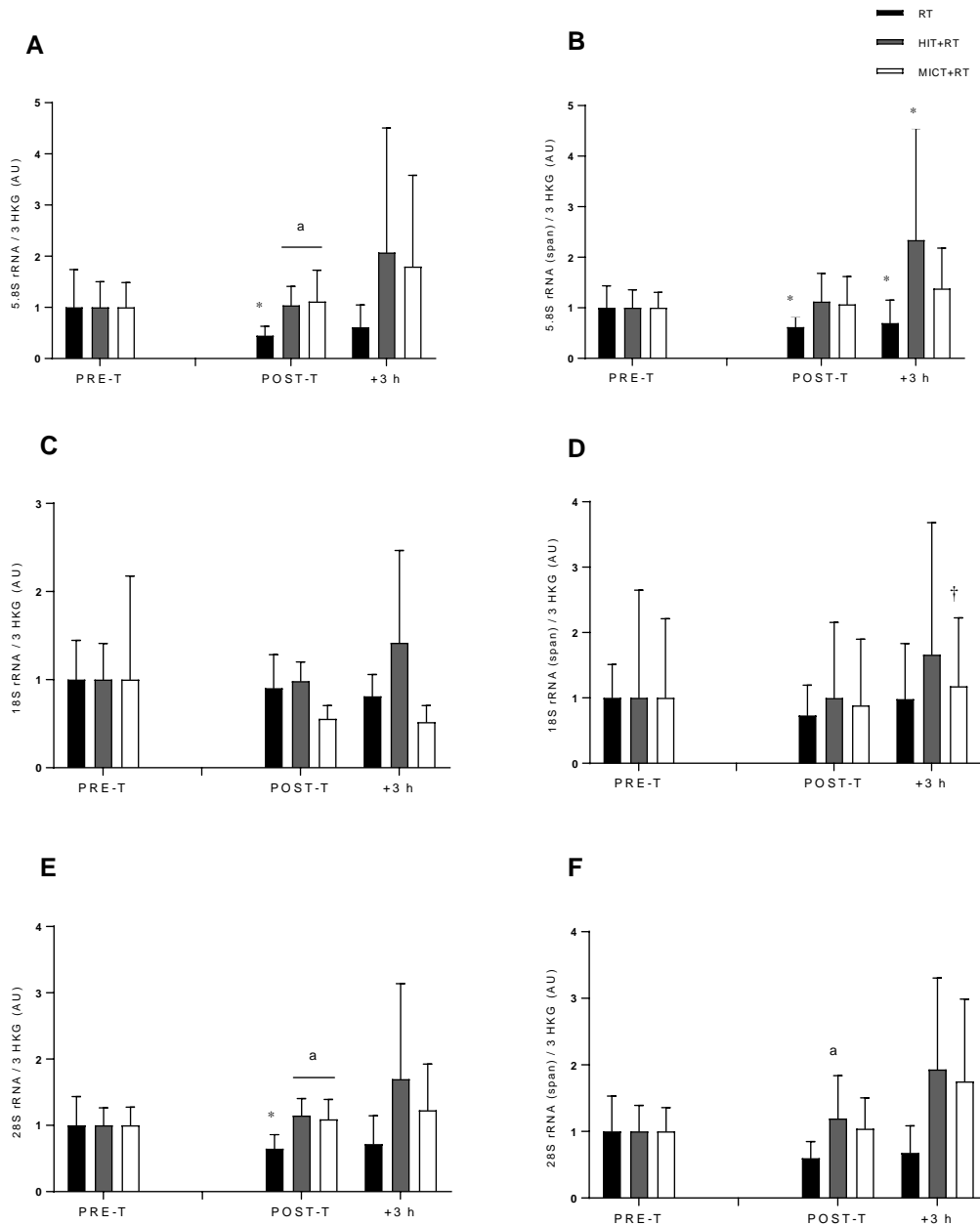


Figure 7. Expression of the mature rRNA transcripts 5.8S rRNA (A), 18S rRNA (C), and 28S rRNA (E), and rRNA transcripts bound to the 45S pre-RNA precursor: 5.8S rRNA (span) (B) 18S rRNA (span) (D) and 28S rRNA (span) (F) relative to the geometric mean of cyclophilin, β 2M and TBP expression before (PRE-T) 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 \pm 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.

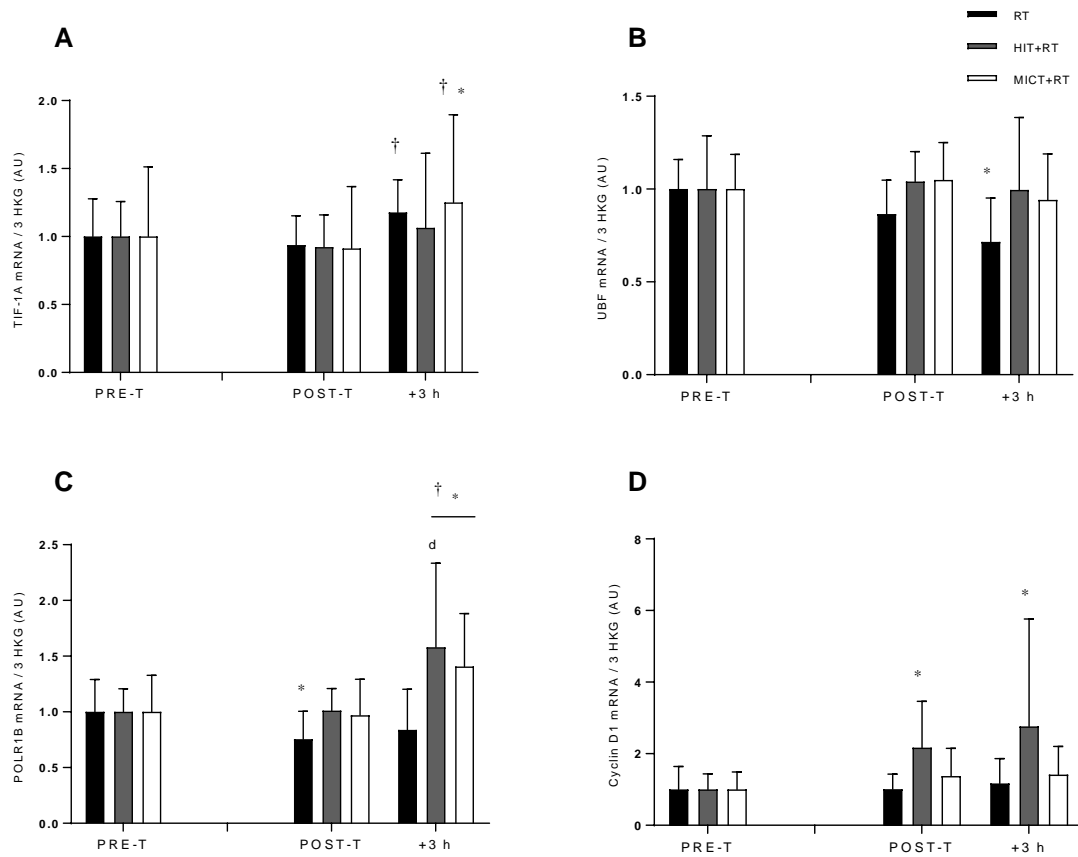


Figure 8. mRNA expression of TIF-1A (A), UBF (B), POLR1B (C), and cyclin D1 (D) relative to the geometric mean of cyclophilin, β 2M and TBP expression before (PRE-T) and after (POST-T) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity continuous training (MICT+RT), and 1 h and 3 h after a single exercise bout performed post-training. Data presented are means \pm 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.

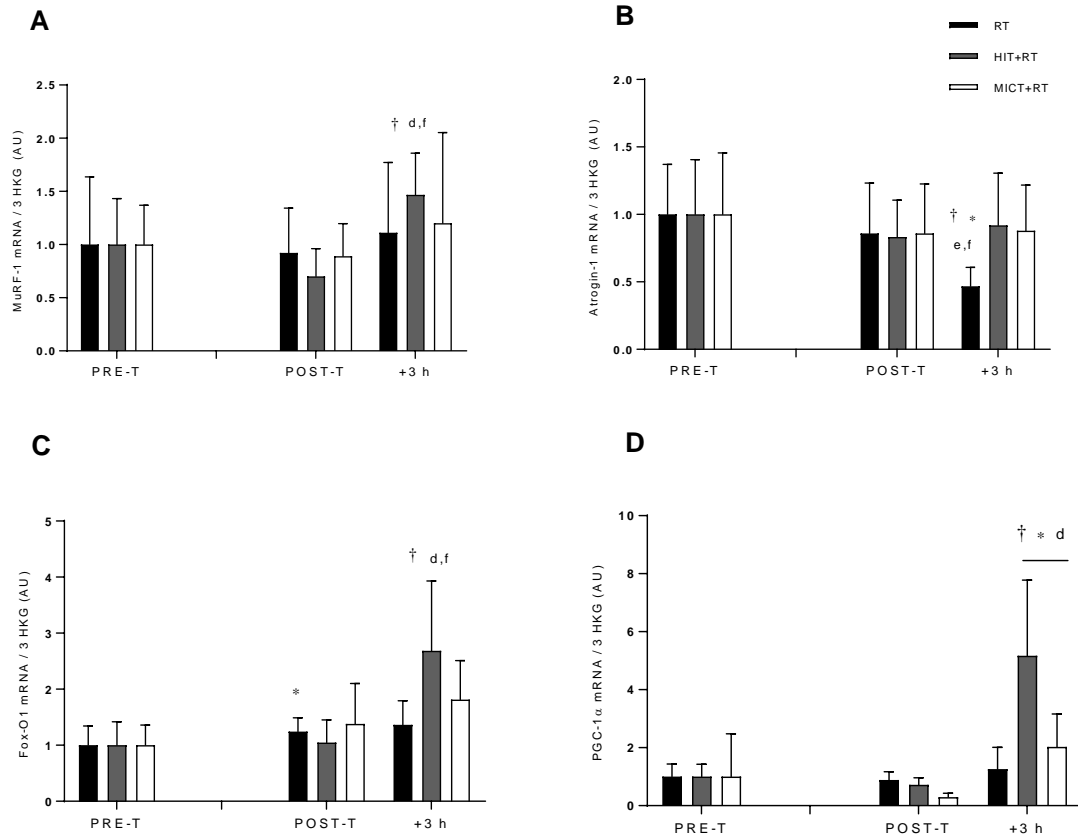


Figure 9. mRNA expression of MuRF-1 (A), Atrogin-1 (B), Fox-O1 (C) and PGC-1α (D) relative to the geometric mean of cyclophilin, β2M and TBP expression before (PRE) and after (POST) eight weeks of either RT alone, or RT combined with either high-intensity interval training (HIT+RT) or moderate-intensity 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.

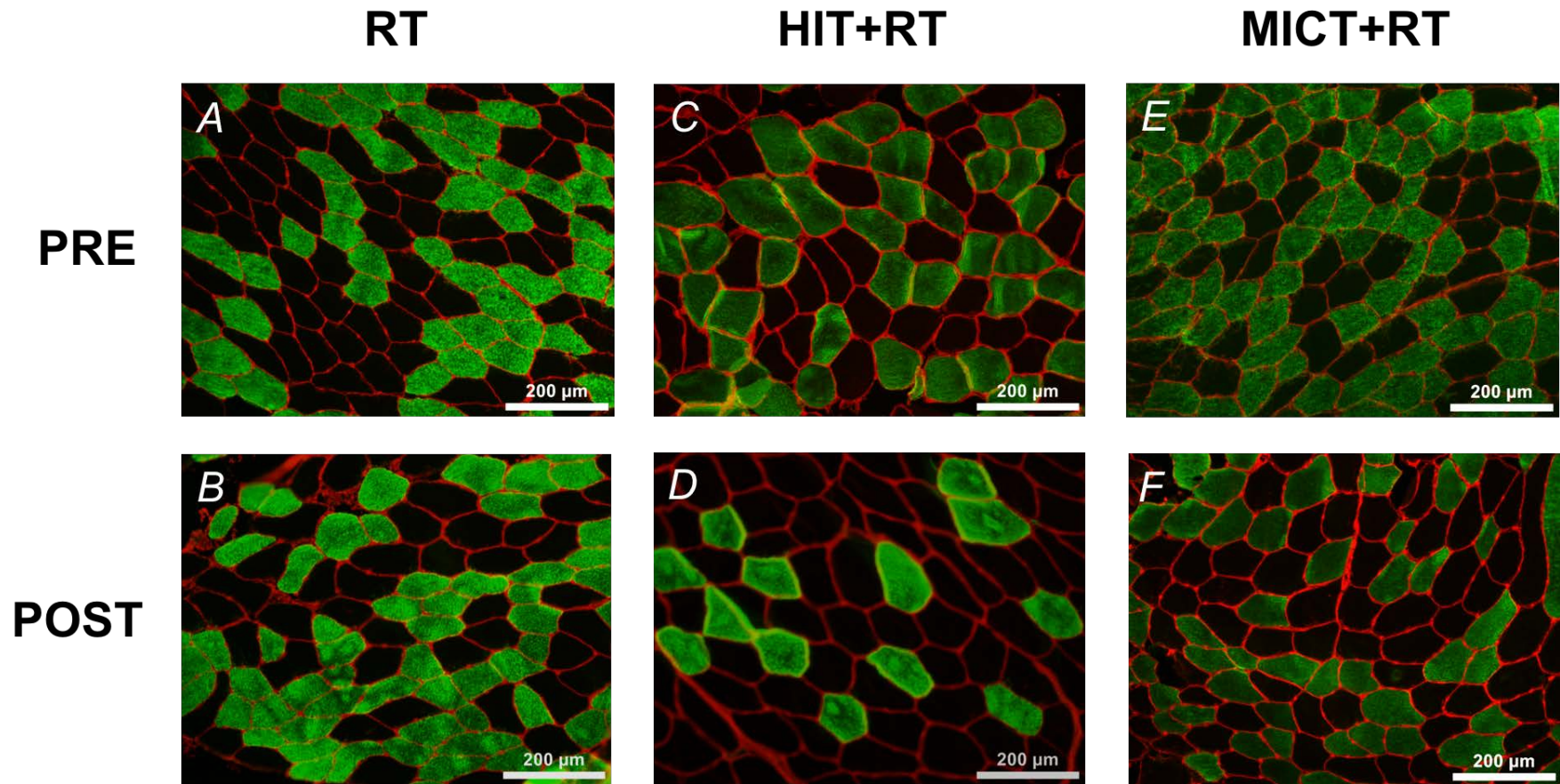


Figure 10. Representative immunohistochemical images of muscle cross-sections obtained before (PRE) and after (POST) eight weeks of either RT alone (images A and B, respectively), or RT combined with either high-intensity interval training (HIT+RT; images C and D, respectively) or moderate-intensity 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.