SATELLITE CELL RESPONSE TO CONCURRENT RESISTANCE EXERCISE AND HIGH-INTENSITY INTERVAL TRAINING IN SEDENTARY, OVERWEIGHT/OBESE, MIDDLE-AGED INDIVIDUALS

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ABSTRACT

Purpose Sarcopenia can begin from the 4-5th decade of life and is exacerbated by obesity and inactivity. A combination of resistance exercise (RE) and endurance exercise is recommended to combat rising obesity and inactivity levels. However, work continues to elucidate whether interference in adaptive outcomes occur when RE and endurance exercise are performed concurrently. This study examined whether a single bout of concurrent RE and high-intensity interval training (HIIT) alters the satellite cell response following exercise compared to RE alone.

Methods Eight sedentary, overweight/obese, middle-aged individuals performed RE only (8x8 leg extensions at 70% 1RM), or RE+HIIT (10x1 min at 90% HR_{max} on a cycle ergometer). Muscle biopsies were collected from the *vastus lateralis* before and 96 h after the RE component to determine muscle fiber type-specific total ($Pax7^+$ cells) and active ($MyoD^+$ cells) satellite cell number using immunofluorescence microscopy.

Results Type-I-specific $Pax7^+$ (P=0.001) cell number increased after both exercise trials. Type-I-specific $MyoD^+$ (P=0.001) cell number increased after RE only. However, an elevated baseline value in RE+HIIT compared to RE (P=0.046) was observed, with no differences between exercise trials at 96 h (P=0.21). Type-II-specific $Pax7^+$ and $MyoD^+$ cell number remained unchanged after both exercise trials (all $P \geq 0.13$).

Conclusion Combining a HIIT session after a single bout of RE does not interfere with the increase in type-I-specific total, and possibly active, satellite cell number, compared to RE only. Concurrent RE+HIIT may offer a time-efficient way to maximise the physiological benefits from a single bout of exercise in sedentary, overweight/obese, middle-aged individuals.

KEYWORDS

Concurrent exercise; resistance exercise; high-intensity interval training; obesity; acute responses; interference; satellite cell; mRNA expression; human skeletal muscle.

ABBREVIATIONS

1RM	One-repetition maximum
BMI	Body mass index ($kg \cdot m^2$)
HIIT	High-intensity interval training
HR_{max}	Heart rate maximum
Myf5	Myogenic factor 5
Myf6	Myogenic factor 6
MyoD	Myogenic differentiation 1
MyoG	Myogenin
Pax7	Paired box transcription factor 7
RE	Resistance exercise
RE + HIIT	Concurrent resistance exercise and high-intensity interval training
RPE	Rate of perceived exertion
SEM	Standard error of mean
$\dot{V}O_{2\ peak}$	Peak oxygen uptake ($mL \cdot kg \cdot min^{-1}$)
W	Watts (W)

INTRODUCTION

Ageing often results in the degenerative loss of significant muscle mass and strength, known as sarcopenia (Bijlsma et al. 2013), a process starting as early as the 4th or 5th decade (Marcell 2003; Jackson et al. 2012). Exercise can lessen the effect of sarcopenia, however, 45% of women and 33% of men do not meet the current physical activity guidelines (Health & Social Care Information Centre 2013). Sarcopenia is further accelerated in the presence of obesity and can result in physical disability and a lower quality of life (Dominguez and Barbagallo 2007; Stenholm et al. 2008) and in England, 58% of women and 65% of men are classified as overweight or obese (Health & Social Care Information Centre 2016). The combination of physical inactivity and obesity underpins a number of chronic diseases (e.g., type 2 diabetes and cardiovascular disease) (Rana et al. 2007; Reddigan et al. 2011), and are considered major global public health issues. Strategies to encourage increased physical activity in these populations, which may in turn reduce obesity, could slow the aging process and development of chronic disease.

Exercise is an effective stimulus for inducing increases in muscle mass, weight loss and cardio-metabolic health irrespective of age, and therefore could play a major role in combatting the fight against the increase in obesity and obesity-related diseases. Current exercise guidelines recommend that middle-aged individuals (~40-65 years) should engage in a combination of endurance and resistance exercise (RE), in order to improve cardio-metabolic health and quality of life (Chief Medical Office 2011; Garber et al. 2011). It is often recommended that individuals should complete five 30 min sessions of moderate-intensity endurance exercise and two sessions of RE per week, therefore requiring up to seven days of exercise engagement per week, which may provide a significant barrier to some.

The design of a concurrent training program incorporating RE and endurance exercise within a single session provides a practical, time-efficient protocol that may be more appealing to individuals, particularly those who are not “natural exercisers”, and therefore increase motivation and adherence. In support of this viewpoint, Larose et al. (2012) implemented a 6 month

concurrent training program with sedentary, overweight/obese, middle-aged individuals with type 2 diabetes. The participants were given the option as to when they performed each exercise component. Remarkably, all participants chose to perform both RE and endurance exercise components within a single session. However, there is evidence to suggest that combining RE and endurance exercise will impair strength development and muscle size (Hickson 1980; Craig et al. 1991; Hennessy and Watson 1994; Coffey et al. 2009a,b; Babcock et al. 2012; Kikuchi et al. 2016; Fyfe et al. 2016), although such evidence is equivocal (Bell et al. 1991; Shaw et al. 2009; Donges et al. 2012; Lundberg et al. 2012, 2013; Apró et al. 2013; Kazior et al. 2016). We have recently shown that using an acute bout of high-intensity interval training (HIIT) in combination with RE, as an alternative to moderate-intensity endurance exercise with RE, does not impede acute (<6 h) gene expression and protein signalling markers of muscle growth compared to a single bout of RE alone in young, healthy individuals (Pugh et al. 2015). Furthermore, concurrent RE + HIIT resulted in greater increases in the expression of PGC-1α mRNA suggesting parallel endurance-type adaptations (Olesen et al. 2010). Thus, an exercise protocol that combines both RE and HIIT, as an alternative form of endurance exercise, into a single session, may help individuals meet current exercise guidelines in a time-efficient manner without compromising RE and endurance exercise-induced adaptations. Although this evidence provides indicative responses, it is unlikely that the mechanism behind the impaired adaptations following concurrent training can be fully explained by the initial molecular interference between the signalling proteins, AMPK and mTOR (Hamilton and Philp 2013).

Satellite cell content has been shown to be correlated with an improvement in cross-sectional area of the quadriceps muscle following RE training (Bellamy et al. 2014). Utilising this methodology, following an acute concurrent exercise protocol of RE plus moderate-intensity endurance exercise Babcock et al., (2012) demonstrated an impairment in satellite cell response after concurrent exercise (-6% change from baseline) compared to RE only (38% increase) in young, healthy males. This disparity in the satellite cell response was reported to occur in a fiber-type-specific manner. There was a suppression in type I muscle fiber-specific satellite cell density four days (96 h) after both endurance exercise (-7%) and concurrent exercise (-8%), compared

to RE only (46% increase). In type II muscle fibers, satellite cell density remained unchanged after endurance exercise and concurrent exercise while increasing after RE only. The authors concluded that concurrent exercise, comprising of RE and moderate-intensity exercise, impairs the acute satellite cell response to single bout of RE, thus implicating that this combination could impede muscle growth.

While an increase in satellite cell response to RE is widely accepted (Crameri et al. 2004, 2007; Dreyer et al. 2006; O'Reilly et al. 2008; McKay et al. 2009; Mikkelsen et al. 2009) the response to endurance exercise is limited and inconclusive. Evidence suggests that exercise intensity, rather than duration, may play a key role in the expansion of the satellite cell content (Kurosaka et al. 2012) with studies using moderate-intensity endurance exercise finding no changes in the satellite cell content (Snijders et al. 2011), whereas, studies using high-intensity exercise have shown an increase (Charifi et al. 2003; Verney et al. 2008; Nederveen et al. 2015). Based on these findings, together with our previous study (Pugh et al. 2015), the present study investigated the fiber type-specific satellite cell response to a single bout of RE immediately followed by a bout of high-intensity interval cycling compared to RE alone in sedentary, overweight/obese, middle-aged individuals. It was hypothesised that there would be no interference in the muscle fiber type-specific satellite cell response when a single bout of HIIT is performed immediately after RE.

METHODS

Participants

Of the 14 participants enrolled in the study, eight sedentary, overweight/obese, middle-aged male (n = 3) and female (n = 5) individuals completed both trials and were included in the analysis (Table 1). A participant flow diagram is reported in Figure 1. Sedentary status was defined as no planned or regular patterns of physical activity or exercise on one or more days per week in the preceding six months. Overweight/obese classification was based on a BMI between 27 and 35

kg·m^2, and the presence of abdominal obesity (male ≥94 cm; female ≥80 cm). All participants provided full written informed consent. Prior to participation, all participants underwent comprehensive medical assessment, including an electrocardiogram and physical examination to confirm that there were no underlying contraindications to exercise and to confirm that all were free from any medication. A capillary blood sample was taken to analyse fasting glucose, triglycerides, total cholesterol and high-density lipoprotein (HDL)-cholesterol (CardioChek, Polymer Technology Systems, Indianapolis, IN, USA). Participants had no history of diabetes, or presence of the metabolic syndrome. Diagnostic criteria for metabolic syndrome were the presence of any three (or more) of the following factors (Alberti et al. 2005): increased waist circumference (male ≥94 cm; female ≥80 cm); raised triglycerides (≥1.7 mmol·L^{-1}); reduced HDL-cholesterol (male <1.03 mmol·L^{-1}; female <1.29 mmol·L^{-1}); raised blood pressure (systolic ≥130 mmHg; diastolic ≥85 mm Hg) and/or raised fasting plasma glucose (≥5.6 mmol·L^{-1}). The local Human Research Ethics Committee approved all study procedures.

*** Table 1 near here ***

*** Figure 1 near here ***

Study design and rationale

A schematic of the study design is displayed in Figure 2. This study adopted a counter-balanced crossover design. In one session participants completed a single bout of resistance exercise (RE) and in the other session participants performed RE followed by a single HIIT session (RE + HIIT), each trial was separated by a minimum of 14 days (range: 14 – 36 days), during which time the participants were instructed to maintain their habitual lifestyle. Preliminary tests (maximal strength and $\dot{V}O_{2\,peak}$ test) were completed followed by a separate session where

participants were familiarised with the RE and HIIT sessions at least two weeks before the first experimental trial.

The current project was designed to determine if HIIT performed immediately after RE impairs the satellite cell response to RE. The exercise order was chosen to maximise the anabolic response following RE, which has previously been shown to be diminished when endurance exercise precedes RE (Coffey et al. 2009a, b). Whereas, as we have previously shown no initial molecular interference on gene expression and protein signalling markers of muscle growth with concurrent RE followed by HIIT compared to RE alone (Pugh et al. 2015). Skeletal muscle biopsies were taken before and 96 h after exercise to capture the peak in the RE-induced satellite cell content (Martin and Lewis 2012; Snijders et al. 2015). The timing of the biopsies also allowed direct comparison to Babcock et al. (2012), which is the only other known study to investigate the effects of a single bout of concurrent exercise on the satellite cell response. The present study used a realistic exercise program in order to elicit an exercise-induced satellite cell response, in comparison to other studies (Crameri et al. 2004; Mikkelsen et al. 2009) using extreme workloads that are unfeasible and result in an exaggerated satellite cell response due to muscle damage. While no measure of muscle damage was made in current study, others using a similar workload have shown that the acute satellite response to a single bout of RE correlates with the degree of muscle hypertrophy following training (Bellamy et al. 2014). Therefore, the acute satellite cell response to a single bout of exercise, irrespectively of the stimuli (exercise-/damage-induced), is still relevant to the potential impact of muscle adaptations to concurrent exercise.

*** Figure 2 near here ***

190 *Preliminary testing*

191 **Maximal strength.** Participants were asked to arrive fasted (at least 4 h) and having avoided
192 any strenuous physical activity 48 h before the preliminary tests. Each participant performed a
193 unilateral one-repetition maximum (1RM) on each leg using a leg extension machine
194 (Technogym, Cesena, Italy). Participants were familiarised with the movement and warmed up
195 prior to testing by performing 6 repetitions (at ~40% of estimated 1RM) and 4 repetitions (at ~60%
196 of estimated 1RM) through a full range of motion with 1 min recovery. After each successful lift,
197 3 min recovery was given, subsequently the weight was increased until a failed attempt occurred.
198 The 1RM was attained within 5 attempts.

199 **$\dot{V}O_{2\ peak}$.** Following a 30 min rest, a continuously ramped $\dot{V}O_{2\ peak}$ test was performed on an
200 electrically braked cycle ergometer (Lode Excalibur, Groningen, Netherlands). After a 5 min warm
201 up at 30 W (females) or 50 W (males), workload progressively increased at 16 $W \cdot min^{-1}$ until the
202 participant reached volitional exhaustion. Oxygen consumption ($\dot{V}O_2$) was obtained through
203 breath-by-breath sampling (Cortex MetaLyzer 3B, Leipzig, Germany) that was calibrated prior to
204 each test using gases of known concentrations (17.10% O_2 and 5% CO_2) and a 3 L Hans Rudolph
205 syringe. $\dot{V}O_{2\ peak}$ was determined as the highest value achieved over an 11 breath average. Heart
206 rate was continuously recorded during the exercise (RS300, Polar, Finland) and participants were
207 asked to maintain a cadence between 80-100 $r \cdot min^{-1}$.

208 *Diet and physical activity control*

209 Participants were instructed to avoid alcohol and caffeine during the 48 h period prior to the
210 two main experimental trials and the 96 h follow-up visit. A physical activity diary and weighed
211 food diary was recorded 48 h and 24 h before and throughout the first experimental trial,
212 respectively. Participants were asked to replicate both physical activity levels and diet prior to
213 each visit in the second experimental trial.

214 *Experimental trials*

215 On the morning of each trial, participants arrived at laboratory at 0800 following an overnight
216 fast (~10 h). A resting skeletal muscle biopsy sample was obtained from the middle portion of the
217 *vastus lateralis* muscle of one leg. The participants then performed either the RE or RE + HIIT
218 session. During both exercise sessions, participants received continuous verbal encouragement.
219 For all trials, rating of perceived exertion (RPE, Category-Ratio 10 Scale) (Borg 1998) was
220 recorded after each set of leg extensions and each 1 min bout of high-intensity cycling.
221 Participants were allowed to consume water *ad libitum* throughout. Following the exercise
222 session, the participant was free to leave and asked to return 4 days later after an overnight fast
223 (~10 h) for a subsequent muscle biopsy taken 96 h after the RE component.

224 *Resistance exercise (RE) protocol*

225 Participants completed a standardised warm up consisting of 2 sets of 8 repetitions of
226 unilateral leg extensions at 30% 1RM, immediately followed by the contralateral leg. This was
227 followed by 8 sets of 8 repetitions at 70% 1RM on each leg. Constant feedback and visual
228 markers were provided in an attempt to match all repetitions for velocity (2-s concentric and
229 eccentric phases) and range. Each set was separated by a 2 min recovery.

230 *Concurrent resistance exercise and high-intensity interval training (RE + HIIT) protocol*

231 Immediately after an RE protocol identical to that described above, participants completed a
232 3 min warm up at 30-50 W on the cycle ergometer. This was followed by the completion of 10
233 repetitions of 1 min cycling at an intensity designed to elicit 90% of their heart rate maximum
234 (HR_{max}), with each repetition separated by 1 min of cycling at 30 W (females) or 50 W (males).
235 Participants were instructed to maintain a cadence between 80-100 $r \cdot min^{-1}$ during each interval.

236 *Muscle biopsies*

237 Skeletal muscle samples were obtained from the middle portion of the *vastus lateralis* muscle
238 using a 5-mm Bergström needle (Dixons Surgical Instruments, Essex, UK) modified with suction.

Four millilitres of local anaesthesia (1% lidocaine) was administrated into the skin and subcutaneous tissue above the muscle belly of the *vastus lateralis*. Upon excision of a specimen, any visible fat and/or connective tissue was removed and excess blood was blotted on filter paper. Immediately after, samples were dissected into pieces that were either snap frozen in liquid nitrogen, or embedded in Tissue-Tek optimum cutting temperature (OCT) compound (Agar Scientific, Essex, UK), immersed in liquid nitrogen-cooled isopentane, and stored at -80˚C until later analysis.

Immunofluorescence microscopy

Muscle cross sections (9 µm) from OCT-embedded tissue were cut at -20˚C using a cryostat microtome (Thermo Scientific, Runcorn, UK), and allowed to air dry for 30 min at room temperature, before being stored at -80˚C until subsequent analysis. Tissue sections were fixed in 4% paraformaldehyde fixing solution for 10 min at room temperature, washed with phosphate-buffered saline (PBS) containing 1% tween-20 (PBST) for 3 x 5 min, and then blocked in PBS containing 2% bovine serum albumin, 2% goat serum, and 0.2% Triton X-100 for 60 min at room temperature. After blocking, sections were incubated with the primary antibodies diluted in PBS blocking solution overnight. Samples were then washed in PBST for 3 x 5 min before secondary antibodies diluted in PSB block were applied and incubated for 2 h. Subsequently, samples were washed in PBST for 4 x 5 min and then covered with a drop of Fluoromount^{TM} aqueous mounting medium (Sigma-Aldrich, Dorset, UK) and a cover slip, and stored in the dark at 4˚C until viewing.

Two serial cross sections were stained; (1) for satellite cell content [paired box transcription factor 7 (Pax7), laminin and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI)], and (2) for the number of active satellite cells [myogenic differentiation 1 (MyoD), laminin and DAPI]. Mouse anti-human antibodies directed against Pax7 [1:200; Developmental Studies Hybridoma Bank (DSHB), Iowa City, IA, USA] and MyoD (clone 5.8A; M351201-2; 1:50; Dako, Burlington, ON, Canada) with goat anti-mouse Alexa Fluor 488-conjugate IgG (A11029; 1:500; Invitrogen, Paisley, UK) secondary antibodies were used to detect quiescent and active satellite cells, respectively. All slides were counterstained with rabbit anti-human antibodies directed against

laminin (AB11575; 1:500; Abcam, Cambridge, MA, USA) with goat anti-rabbit Alexa Fluor 647-conjugate IgG (A21245; 1:1000; Invitrogen) secondary antibodies to detect cell border, and DAPI (F4680; 1:20000; Sigma-Aldrich) to reveal myonuclei.

Double staining of all samples was performed to determine muscle fiber type-specific localisation of satellite cells. Samples were washed in PBST for 4 x 5 min, re-fixed, and blocked. Mouse anti-human antibodies directed against myosin heavy chain I (MHC I; A4.951; 1:1000; DSHB) with goat anti-mouse Alexa Fluor 488-conjugate IgG secondary antibodies were used to detect type I muscle fiber isoforms. Rabbit anti-human antibodies directed against MHC II (AB91506; 1:2000; Abcam) with goat anti-mouse Alexa Fluor 647-conjugate IgG secondary antibodies were used to detect type II muscle fiber isoforms.

Imaging and quantification

Images were viewed at 20x magnification (Leica DM2500, Leica Microsystems, Milton Keynes, UK) and captured with a digital camera (Leica DFC360 FX, Leica Microsystems). At least 50 type I and 75 type II muscle fibers were counted to ensure accurate assessment of the muscle fiber-specific satellite cell content (Mackey et al. 2009). In this study, 63 ± 4 type I and 92 ± 6 type II muscle fibers were evaluated for each muscle biopsy per participant. Image processing and quantitative analyses were completed using ImageJ version 1.49 software (Schneider et al. 2012). All quantitative analyses were conducted in a blinded fashion to the participant coding and experimental trial. The identification of $Pax7^+$ and $MyoD^+$ cells were determined by the co-localisation of either Pax7 or MyoD with DAPI, and located at the periphery of each muscle fiber. Double stained muscle cross sections (first stained for satellite cell and then fiber type) were superimposed to determine the satellite cell response in a muscle fiber-specific manner. This is important as previous studies have shown changes in RE-induced satellite cell response occur in a muscle fiber type-specific manner (Snijders et al. 2012; Cermak et al. 2013).

290 *RNA extraction and reverse transcription*

291 Skeletal muscles samples were homogenised at 20 Hz for 2 x 3 min using a TissueLyser II
292 (Qiagen, Limbury, Netherlands) in 1.0 mL of ice-cooled TRI Reagent (Sigma-Aldrich). Following
293 centrifugation at 13,000 x *g* for 15 min at 4˚C the supernatant was incubated for 5 min at room
294 temperature. Next, 200 µL of chloroform was added and mixed for 20 s then allowed to stand for
295 a further 10 min at room temperature before centrifugation. The upper, clear, aqueous phase
296 containing total RNA was mixed with one volume of isopropanol and incubated for 30 min at room
297 temperature before further centrifugation. The RNA pellet was washed in 1.0 mL of ice-cooled
298 70% ethanol, centrifuged at 7,500 x *g* for 5 min and then repeated, before air-drying. Precipitated
299 RNA was then re-suspended in nuclease-free water. One microliter of each RNA sample was
300 analysed on a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Rockford, IL, USA)
301 to determination RNA concentration and purity. RNA concentration was 166.1 ± 18.3 $ng \cdot \mu L^{-1}$,
302 and the A_{260}/A_{280} ratio, as a measure of purity was 1.86 ± 0.06. An Agilent 210 Expert Bioanalyser
303 with RNA 6000 Nano LabChip kits (Agilent Technologies, Palo Alto, CA, USA) was used to
304 analyse the size and distribution of extracted RNA molecules. An RNA Integrity Number (RIN)
305 was calculated for all samples based on the RIN algorithm of the Agilent 2100 Expert software
306 (version B.02.08). The RIN was 6.6 ± 0.2. Reverse transcription of 20 µL of cDNA was performed
307 using 1 µg of RNA with a high-capacity RNA-to-cDNA kit (Invitrogen). The cDNA samples were
308 then stored at -20˚C until further analysis.

309 *Real-time quantitative Polymerase Chain Reaction (PCR)*

310 Real-time quantitative PCR was performed on a ViiA 7 real-time PCR system (Applied
311 Biosystems, Forest City, CA, USA) under the following PCR cycle conditions; 50˚C for 2 min +
312 95˚C for 10 min + ((95˚C for 15 s + 60˚C for 1 min) x 40 cycles). PCR reactions with 2 x TaqMan
313 Universal Master Mix II with UNG (Invitrogen) and 20 x TaqMan Gene Expression assays
314 (Invitrogen) according to the manufacturer's instructions were used to determine mRNA
315 expression levels for myogenic differentiation 1 (MyoD1, Hs00159528_m1), myogenic factor 5
316 (Myf5, hs00929416_g1), myogenin (MyoG, Hs00231167_m1), myogenic factor 6 (Myf6,

Hs01547104_g1), myostatin (Hs00976237_m1) and β-2-microglobulin (β2M, Hs00984230_m1). In addition, PCR reactions with 2 x SYBR Green JumpStart Taq Ready Mix (Sigma-Aldrich), forward and reverse primers (Sigma-Aldrich) at 500 nmol·L^{-1} were used to determine the mRNA expression levels for β-actin (Primer Design, Southampton, UK) and DNA topoisomerase 1 (TOP1, Primer Design). A melt curve was run on all SYBR Green PCR reactions to assess the amplification specificity. All samples were run in triplicate, and all samples from each participant were run together on the same plate to allow for relative comparison. Data were analysed by cycle threshold values, calculating relative expression using the $2^{-\Delta\Delta CT}$ method. Gene expression was normalised using the geometric mean of three reference genes (β2M, β-actin, TOP1).

Statistical analysis

Data were analysed using IBM SPSS version 22 statistical software (IBM Corp., Armonk, NY, USA). All outcomes were examined using linear mixed models with repeated-measures and each participant as a random effect. This statistical model allows use of all available data, while avoiding imputation of missing data (RE at baseline in one male participant). A linear mixed model was used to examine differences in RPE responses with exercise trial included as a fixed factor. Changes in satellite cell content ($Pax7^+$ cells), active satellite cell number ($MyoD^+$ cells), mRNA expression and muscle fiber type distribution were analysed using a linear mixed model with time and exercise trial included as fixed effects. Muscle fiber types were analysed separately. When an interaction was identified a pairwise multiple comparisons with a Bonferroni correction was used to locate differences. Differences in all data sets were considered statistically significant at a two-tailed critical level of $P<0.05$. Data are expressed as mean ± standard error of mean (SEM). A *priori* sample size calculation was performed using G*Power software (Version 3.1.7; Faul et al. 2007). Based on previously published data (Babcock et al. 2012), it was determined that a sample size of six participants would be necessary. This sample size would allow detection of a mean change of 0.024 in satellite cell content ($Pax7^+$) per muscle fiber. Sample size calculation was performed with an alpha error of 0.05, and a power of 80%.

RESULTS

Exercise trial responses

All participants completed the same number of sets and repetitions (8 sets x 8 repetitions at 70% 1RM). The RE workload was 38.8 ± 2.0 kg for males and 21.3 ± 2.5 kg for females. The HIIT workload during the 60 s effort was 245 ± 38 W for males and 120 ± 16 W for females. Heart rate during HIIT intervals corresponded to 90 ± 2% of HR_{max}. No differences for RPE scores (all $P \geq 0.11$) were observed between exercise components in both trials with RE and RE + HIIT being rated as equally strenuous [RE only, 6.2 ± 0.6; RE + HIIT, 6.2 ± 0.6; (RE component, 6.7 ± 0.6; HIIT component, 5.6 ± 0.5)].

Muscle fiber characteristics

Muscle fiber composition was 40.4 ± 2.6% type I and 59.6 ± 2.5% type II muscle fibers. There were no statistical differences observed in fiber type distribution between trials or across time (all $P \geq 0.10$).

Satellite cell content ($Pax7^+$ cells)

Representative immunofluorescent images are shown in Figure 3A-D. There was a main effect of time ($P=0.001$), but no main effect of trial ($P=0.73$), or an interaction effect ($P=0.45$) for satellite cell content ($Pax7^+$ cells) per type I muscle fiber (Figure 3E). Muscle fiber type-I-specific satellite cell content increased (78 ± 24%) at 96 h compared to baseline following both exercise protocol. There were no main effects of time ($P=0.71$), trial ($P=0.36$), or an interaction effect ($P=0.98$) in satellite cell content per type II muscle fiber (Figure 3F).

*** Figure 3 near here ***

Number of active satellite cells (MyoD$^+$ cells)

Representative immunofluorescent images are shown in Figure 4A-D. There was a main effect of time (P=0.006) and an interaction effect (P=0.025), but no main effect of trial (P=0.53), in the number of active satellite cells (MyoD$^+$ cell) per type I muscle fiber (Figure 4E). Post hoc analysis revealed an increase (P=0.001) in muscle fiber type-I-specific MyoD$^+$ cells at 96 h compared to baseline following RE. Conversely, muscle fiber type-I-specific MyoD$^+$ cells remained unchanged over time for RE + HIIT (P=0.64). There was no difference (P=0.21) between exercise trials at 96 h. However, RE + HIIT demonstrated an elevated (P=0.046) baseline value compared to RE, which may have impacted the RE + HIIT exercise response. There was a main effect of trial (P=0.049), but no main effect of time (P=0.13), or an interaction effect (P=0.96) in MyoD$^+$ cell per type II muscle fiber (Figure 4F). There was an overall higher number of MyoD$^+$ cell per type II muscle fiber in RE as compared to RE + HIIT.

*** Figure 4 near here ***

Intramuscular mRNA expression

There were no main effects of time (all P≥0.49), trial (all P≥0.38) or an interaction effect (all P≥0.39) for the expression of MyoD, Myf5, MyoG, Myf6 and myostatin mRNA (Figure 5).

*** Figure 5 near here ***

DISCUSSION

The aim of this study was to establish the effect of a single bout of concurrent RE + HIIT compared to an isolated RE session on the total and active number of satellite cells at rest and 4 days (96 h) after exercise in sedentary, overweight/obese, middle-aged individuals. For the first time, it is shown that both a single bout of RE and concurrent RE + HIIT results in an increase in satellite cell content in type-I-specific muscle fibers, with no difference between exercise regimens. In addition, there was no difference in the number of active ($MyoD^+$ cells) type-I-specific satellite cells at 96 h after both exercise trials. The current findings imply that concurrent RE + HIIT does not compromise the transient RE-induced increase in total satellite cell content, and possibly the number of active satellite cells, after 96 h in sedentary, overweight/obese, middle-aged individuals. Therefore, concurrent RE + HIIT exercise programmes may offer a potent, time-efficient exercise strategy, which could help those that may not be "natural exercisers" meet the current exercise guidelines.

Expansion in satellite cell content peaks between 72-96 h following exercise, and declines thereafter (Martin and Lewis 2012, Snijders et al. 2015). The present study demonstrated a 78 ± 24% increase in the satellite cell content associated with type I muscle fibers 96 h after both exercise protocols. While previous exercise protocols using higher workloads have elicited greater increases (>95%) in satellite cell content (Crameri et al. 2004; O'Reilly et al. 2008; McKay et al. 2009; Mikkelsen et al. 2009), the 78 ± 24% increase in the present study is comparable with data where similar workloads were used (Babcock et al. 2012; Snijders et al. 2014). The RE used in the current study was designed to represent a realistic, exercise program for untrained and overweight/obese individuals, and therefore characterises a real-life exercise-induced stimulus.

Consistent with the present study, an expansion in satellite cell content following a single bout of RE has been reported in both young and older adults (Martin and Lewis 2012; Snijders et al. 2015), and at least in the young, has been shown to be important in determining changes in muscle mass to chronic exercise training (Bellamy et al. 2014). While it remains to be determined

415 if this is true in middle-aged and older adults, an exercise program resulting in a potent expansion
416 of satellite cell content is likely to help reduce muscle mass loss with ageing. The satellite cell
417 content associated with type I fibers has been shown to acutely increase following RE in older
418 adults. However, in type II fibers this response is blunted (Snijders et al. 2014), or fails to respond
419 entirely (McKay et al. 2012; Nederveen et al. 2015). An attenuated decline in myostatin
420 colocalisation with satellite cells has been proposed as one mechanism for this blunted response
421 in older adults (McKay et al. 2012; Snijders et al. 2014). Similarly, the current study demonstrates
422 that both exercise programs resulted in an increase in type I associated satellite cell content, but
423 no expansion in the satellite cell content was noted with type II fibers in sedentary,
424 overweight/obese, middle-aged individuals. It should be noted that the lack of satellite cell
425 response in type II muscle fibers could merely be reflective of the timing of the final biopsy.

426 The data from the current study suggest that concurrent RE + HIIT does not impair the
427 elevation in satellite cell content, particularly in type I fibers, 96 h after a single bout of RE, which
428 is in contrast to a similar study that employed moderate-intensity continuous endurance exercise
429 instead of HIIT (Babcock et al. 2012). However, rodent studies have suggested that the increase
430 in satellite cell content is related to exercise intensity rather than duration (Kurosaka et al. 2012).
431 In humans, two recent studies implementing similar HIIT protocols to the current study have
432 demonstrated that HIIT could offer a greater hypertrophic stimulus than moderate-intensity
433 endurance exercise in sedentary older men (Bell et al. 2015; Nederveen et al. 2015). Similarly,
434 an increase in lean mass in leg and groin regions has been observed following 6 weeks of HIIT
435 in overweight women (Gillen et al. 2013). The anabolic potential of HIIT therefore raises the
436 hypothesis that incorporating HIIT, rather than moderate-intensity continuous endurance
437 exercise, concurrently with RE, may act to abolish the interference effect between the different
438 adaptive responses.

439 The satellite cell data is supported by that of the myogenic regulatory factor (MRF) MyoD,
440 which represents an important marker of satellite cell activity. MyoD is expressed during
441 activation, proliferation and during the early stages of differentiation, but not in quiescent satellite

442 cells (Zammit et al. 2004). In the present study, the increase in the number of MyoD$^+$ cells is
443 similar to that previously reported after a single bout of RE or HIIT (Nederveen et al. 2015).
444 Specifically, the type I associated MyoD$^+$ cell number showed no difference between exercise
445 trials at 96 h, reflecting the increase in satellite cell content at 96 h in both exercise trials.
446 However, no change in type I associated MyoD$^+$ cell number was reported from baseline to 96 h
447 in RE + HIIT, whereas RE demonstrated an increase. This is likely due to the reported high
448 baseline value in RE + HIIT compared to RE, which may have masked any change from baseline.
449 The reason for this difference at baseline is unknown. Furthermore, the reason for the overall
450 difference in muscle fiber type-II-specific MyoD$^+$ cell number between exercise trials is unknown.
451 Irrespective of this, muscle fiber type-II-specific MyoD$^+$ cell number remained unchanged across
452 time in both exercise trials with no evidence of an interference of concurrent RE + HIIT. It is worth
453 noting that in the current study there were no significant differences in satellite cell content
454 following RE or RE + HIIT despite a greater workload completed in the concurrent exercise trial.
455 While this study clearly demonstrates that RE + HIIT does not affect the RE-induced satellite cell
456 response, it does raise the question as to whether the magnitude of the satellite cell response
457 could be further increased if the concurrent exercise strategy is optimised.

458 Myostatin is known to be a negative regulator of muscle growth (McPherron and Lee 1997;
459 Reisz-Porszasz et al. 2003) through the suppression of muscle protein synthesis and satellite
460 cell activity (Langley et al. 2002; McCroskery et al. 2003; Welle et al. 2009). A single bout of RE
461 or endurance exercise, either in isolation or in combination, has been shown to decrease
462 myostatin mRNA expression (Louis et al. 2007; Lundberg et al. 2012). While the present study
463 showed no statistical change in myostatin mRNA expression after either exercise trial, we have
464 previously demonstrated a downregulation in myostatin mRNA after exercise (<6 h) with no
465 differences between RE and RE + HIIT (Pugh et al. 2015). The lack of consistency could be
466 explained by timing of the muscle biopsy. No changes in mRNA expression of the MRFs (MyoD,
467 Myf5, MyoG and Myf6) were found at 96 h compared to baseline after either exercise trial. Others
468 have reported increases in MyoG and Myf6 mRNA expression up to 120 h after high-volume,
469 muscle damaging RE (McKay et al. 2008). However, it is likely that less damaging exercise

results in earlier (<96 h) peaks in mRNA expression of MRFs (Psilander et al. 2003; Yang et al. 2005). Whilst the present study shows no change in MyoD mRNA expression, the increase in $MyoD^+$ cells may suggest that the increases in transcription have already occurred within the 96 h timeframe. Future studies are warranted to investigate the temporal response of myostatin and MRFs expression, both at the gene transcription and protein level, on the effect of satellite cell regulation in response to concurrent exercise.

Given that HIIT improves the satellite cell response, but also has profound health benefits across both healthy (Trapp et al. 2008; Gillen et al. 2013; Faulkner et al. 2015) and clinical populations (Currie et al. 2013; Madsen et al. 2015; Cassidy et al. 2016) matching or exceeding that of traditional endurance exercise (Tjønna et al. 2008; Kemmler et al. 2015), it would suggest that this form of higher intensity activity should be the preferred option for concurrent exercise regimens. In addition, HIIT has also been described as a time-efficient alternative (<25 min) to more traditional moderate-intensity exercise (Gibala et al. 2012), with reports of high exercise adherence (Terada et al. 2013; Currie et al. 2013; Faulkner et al. 2015) and enjoyment (Bartlett et al. 2011). The data from the present study indicates that incorporating HIIT after RE does not dampen the increase in satellite cell content following a single bout of RE, while potentially providing the stimulus for important cardiovascular and metabolic adaptations previously attributed to HIIT (Gibala et al. 2012). However, acute exercise studies only provide a framework, and therefore further work into the long-term consequences of chronic concurrent RE and HIIT on the satellite cell response compared to RE training only is warranted.

Practical implications of concurrent training

Often a lack of time is cited as the main barrier to an individual participating in regular physical activity (Stutts 2002; Trost et al. 2002). This is particularly true when both RE and endurance exercise components are required. Scheduling each exercise component to occur within a single session, such as with concurrent training, has been shown to be the preferred option for individuals with type 2 diabetes (Larose et al. 2012). This illustrates the importance of minimising the number of training visits, together with overall time commitment. Specifically, the concurrent

RE + HIIT program used here was completed within a single exposure minimising both the exercise time commitment (HIIT vs. moderate-intensity continuous endurance exercise: 75 vs. 150 min), and the number of training sessions per week (concurrent RE + HIIT vs. individual exercise sessions: three vs. six sessions). This study has demonstrated the feasibility of this concurrent RE + HIIT model in sedentary, overweight/obese, middle-aged individuals, which may provide these individuals with an alternative strategy to increase their regular physical activity levels. However, future studies are warranted to determine the chronic effectiveness of this concurrent RE + HIIT program.

Limitations of the study

A potential limitation of the study is that the total work executed was higher in RE + HIIT compared to RE. It is plausible that any interference effect could have been masked with the results reflecting differences in contractile activity, rather than the exercise-mode (i.e. HIIT). However, this study set out to examine the interference effect to a practical/realistic exercise model that could be applied to the general population, and therefore a prolonged RE-only session would not have been appropriate. Additionally, the sample size in the current study is limited. However, the data indicate clear beneficial effects of concurrent training on acute satellite cell function despite such a small sample size. Finally, in the current study there were different proportions of men and women, which may have led to gender bias. No statistical analysis was performed for sex differences in the present study due to a limited sample size. However, descriptively there were no gender effects. Similarly, others have shown no gender effects when using a mixed gender model (Fry et al. 2014).

Conclusion

For the first time, we have shown that concurrent RE + HIIT does not inhibit the increase in the satellite cell content, and possibly active satellite cell number, arising from a single bout of RE. Concurrent RE + HIIT may offer a time-efficient way to maximise the physiological benefits from a single bout of exercise in sedentary, overweight/obese, middle-aged individuals. Future

studies are warranted to determine if long-term concurrent RE + HIIT affect muscle strength and growth adaptations compared to RE training in isolation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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778 **TABLES**

Table 1. Participants' characteristics

Measure	All (*n* = 8)	Males (*n* = 3)	Females (*n* = 5)
Age (y)	48.4 ± 3.9	52.0 ± 0.1	47.7 ± 5.7
Height (m)	1.73 ± 0.03	1.82 ± 0.06	1.68 ± 0.02
Mass (kg)	93.0 ± 4.7	103.4 ± 8.4	86.8 ± 5.6
BMI ($kg \cdot m^2$)	30.8 ± 0.9	31.2 ± 0.7	30.6 ± 1.4
Waist circumference (cm)	97.3 ± 2.9	105.3 ± 6.1	92.5 ± 2.6
Systolic BP (mmHg)	120 ± 6	126 ± 9	116 ± 7
Diastolic BP (mmHg)	75 ± 3	75 ± 1	76 ± 4
Glucose ($mmol \cdot L^{-1}$)	5.6 ± 0.3	6.3 ± 0.4	5.1 ± 0.1
Total cholesterol ($mmol \cdot L^{-1}$)	5.34 ± 0.41	4.88 ± 0.30	5.61 ± 0.59
HDL-cholesterol ($mmol \cdot L^{-1}$)	1.57 ± 0.10	1.36 ± 0.24	1.69 ± 0.07
Triglycerides ($mmol \cdot L^{-1}$)	1.22 ± 0.13	1.15 ± 0.17	1.26 ± 0.19
$\dot{V}O_{2\ peak}$ ($mL \cdot kg^{1} \cdot min^{-1}$)	25.7 ± 2.6	33.6 ± 4.5	20.9 ± 1.5
Leg extension 1RM (kg)	40.6 ± 5.2	57.1 ± 2.1	30.8 ± 3.5

Data presented as mean ± SEM. 1RM, one-repetition maximum; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; $\dot{V}O_{2\ peak}$, peak oxygen uptake.

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FIGURE CAPTIONS

Figure 1.

Participant flow diagram. The dashed box indicates the participants who withdrew from the study. Following screening one was excluded because they did not meet the criteria. Three participants withdrew prior to the main trials for unknown reasons. One participant discontinued between the exercise visit and 96 h follow-up visit of the participant's first experimental trial for unknown reasons. One participant withdrew following completion of the first trial and did not progress to the second trial due to restricted leg movement. The participant fully recovered. RE, resistance exercise trial; RE + HIIT, resistance exercise and high-intensity interval training trial.

Figure 2.

Schematic diagram of the experimental trials. This study adopted a counterbalanced crossover design where participants completed both exercise trials on separate occasions. RE, resistance exercise trial; RE + HIIT, resistance exercise and high-intensity interval training trial. Arrows indicate sampling time points for muscle biopsies.

Figure 3.

Satellite cell content ($Pax7^+$) before and 96 h after a single bout of resistance exercise (RE) versus resistance exercise and high-intensity interval training (RE + HIIT). (A-D) representative images of muscle fiber type-specific Pax7 immunofluorescent staining. Merged images of (A) Pax7/DAPI/laminin/MHC I (green)/MHC II (red), and (B) Pax7/DAPI/laminin (red) are provided, with single channel views of (C) DAPI (blue) and (D) Pax7 (green). Arrow denotes a $Pax7^+$ cell. Scale bar = 20μm. $Pax7^+$ cells per (E) type I and (F) type II muscle fiber before and 96 h after resistance exercise in both trials. Symbols above lines denote differences when a main effect was observed. * $P<0.05$ vs. Pre. Data presented as mean ± SEM.

Figure 4.

Number of active satellite cells ($MyoD^+$ cells) before and 96 h after a single bout of resistance exercise (RE) versus resistance exercise and high-intensity interval training (RE + HIIT). (A-D) representative images of muscle fiber type-specific MyoD immunofluorescent staining. Merged images of (A) MyoD/DAPI/laminin/MHC I (green)/MHC II (red), and (B) MyoD/DAPI/laminin (red) are provided, with single channel views of (C) DAPI (blue) and (D) MyoD (purple). Arrow denotes a $MyoD^+$ cell. Scale bar = 20μm. $MyoD^+$ cells per (E) type I and (F) type II muscle fiber before and 96 h after resistance exercise in both trials. Symbols above lines denote differences when a main effect was observed. Symbols without lines denote differences revealed by a post-hoc test when an interaction effect was observed * $P<0.05$ vs. Pre; $^{\#}$ $P<0.05$ vs. RE. Data presented as mean ± SEM.

Figure 5.

mRNA expression of (A) MyoD, (B) Myf5, (C) MyoG, (D) Myf6 and (E) myostatin before and 96 h after a single bout of resistance exercise (RE) versus resistance exercise and high-intensity interval training (RE + HIIT). Data presented as mean ± SEM.