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Abstract

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

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

Results: Lean mass increased after RET and HIIT (+3.2±1.6% and +1.6±2.1%, P<0.05). Muscle strength (sum of leg press, leg extension and bench press 1RMs) increased after all training interventions (RET: +25±5%; HIIT: +10±5%; ENT: +7±7%, P<0.05). Aerobic capacity increased only after HIIT and ENT (+14±7% and +11±11%, P<0.05). Type I and II muscle fiber size increased for all groups post-training (main effect of time, P<0.05). Following a period of detraining, the gains in lean mass and maximal muscle strength were maintained in RET and HIIT groups, but maximal aerobic capacity declined below post-training levels in HIIT and ENT (P<0.05).
Conclusion: Six weeks of HIIT induced widespread adaptations prior to detraining in middle-age men. Exercise training-induced increases in aerobic capacity declined during 2.5 weeks of detraining but gains in lean mass and muscle strength were maintained.

Key words: Skeletal muscle growth; muscle adaptation; protein; short-term training
Introduction

Exercise training enhances physical fitness (muscle strength and aerobic capacity) resulting in marked improvements in metabolic health and functional capacity (1). As such, intense exercise training prior to a period of forced or planned inactivity (i.e., injury or postsurgery) is a common practice as a strategy to augment pre-operative physical fitness and post-operative recovery (2-4). Pre-operative exercise training prescription is based upon general exercise training guidelines emphasising combined endurance and resistance exercise training (5). However, whether implementation of both exercise modalities reflects optimal programming for all pre-operative settings is questionable (6, 7).

While combined exercise training can induce robust changes in muscle strength and aerobic capacity over extended time periods (≥12 weeks) (8), many individuals have to undergo surgery at short notice, and do not have time to undertake such exercise training regimens (4). As such, it is important to determine the short-term, mode-specific effects of exercise training on multiple components of physical fitness. Defining short-term muscle adaption responses to different exercise training modalities in healthy middle-age adults is important to provide evidence-based guidelines for short-term pre-operative exercise training programming.

Six weeks of aerobic-based exercise training increases aerobic capacity (9-12). However, the short-term effects of such training interventions on muscle strength are less clear. Another important aspect of physical fitness is skeletal muscle mass which is necessary for mobility as well as whole-body glycaemic control (13, 14). Individuals with low muscle mass before surgery are at increased risk of adverse post-operative outcomes such as major surgery-related
complications, prolonged hospital length of stay, morbidity and mortality (15-18). Yet, comparisons between short-term exercise training modalities on markers of whole-body and regional muscle mass in middle-age adults are lacking.

Another knowledge gap regarding muscle adaptation responses are the effects of short-term exercise training cessation (i.e. detraining) subsequent to divergent exercise training modalities. Detraining is the partial or complete loss of exercise training-induced adaptations due to a reduction or cessation in exercise frequency, intensity or duration (19). In the early post-operative period (i.e. the first few weeks following surgery), exercise training may not be feasible due to pain, nausea or physical restrictions (20). As little as two weeks of reduced physical activity can induce catabolic events in skeletal muscle, resulting in decreased muscle mass (21, 22) and impaired glycaemic control (23). Whether short-term exercise training adaptations are maintained after a short detraining period in middle-age adults is unknown. Ultimately, clarification of short-term single-mode exercise training and detraining responses in healthy middle-age adults will help to inform pre-operative exercise training programming, particularly for populations tasked with time constraints prior to surgery.

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

**Methods**

**Participants & ethics approval**

Thirty-nine males (age 39 ± 3 years; body mass 94 ± 13 kg; body mass index [BMI] 29 ± 3 kg·m⁻²), who were not meeting current national physical activity guidelines (26) for the six months prior to recruitment, volunteered to participate in this study. As four participants withdrew from the study, a total of 35 participants completed the protocol and were included for analysis (Figure 1). All participants completed the *Exercise & Sports Science Australia Adult Pre-exercise Screening Tool* to identify individuals who may be at a risk of an adverse event while exercising, in which case clearance to participate was sought from a medical practitioner prior to participation. Exclusion criteria included: BMI <25 or >35 kg·m⁻², smoking, type 2 diabetes mellitus, regular use of non-steroidal anti-inflammatory medication, scheduling conflicts prohibiting morning exercise session attendance and previous injuries exacerbated by exercise. Written and informed consent was obtained from all participants included in the study. The study was approved by the Australian Catholic University Human Research Ethics Committee (#2017-104H), prospectively registered online (ACTRN12617000894392; 19/06/2017) and conducted in accordance with the most recent revisions of the Declaration of Helsinki. The study was undertaken at the exercise physiology laboratories at the Australian Catholic University’s St Patrick’s campus (Fitzroy, VIC, Australia).
Study design and overview

The study was conducted in a parallel groups design. Participants reported to the laboratory on ten occasions for study measures and a further 18 occasions for supervised exercise training sessions (Figure 2). Allocation to one of the three exercise training groups was based upon enrolment date following completion of all preliminary testing (Pre) using randomised stratification (see Document, Supplemental Digital Content 1, Appendix). During the six weeks of exercise training, participants performed three sessions per week in the morning on alternate days (e.g. Monday, Wednesday and Friday) of either cycling endurance exercise training (ENT), cycling high-intensity interval training (HIIT) or whole-body resistance exercise training (RET).

Dietary recommendations were implemented by prescribing each participant with an energy intake (kJ·d⁻¹) range (resting energy expenditure × physical activity factor of 1.5) (27) and protein (~1.4 g·kg BW⁻¹·d⁻¹) intake target. A study nutritionist monitored adherence to the study dietary prescription via face-to-face diet consultations every fortnight for the entire study, daily electronic dietary recordings and weekly reminders. After six weeks of exercise training, all measurements were repeated (Post). Thereafter, participants were instructed to refrain from exercise training and maintain their activities of daily living for 2.5 weeks after which all measurements were repeated for the final time (detraining; DT).

Initially, participants reported to the laboratory in an overnight fasted state between 0630-0730 h where a dual-energy X-ray absorptiometry scan (DXA; GE Lunar iDXA Pro, enCORE software Version 16, General Electric, Boston, MA, USA) was conducted (28) to assess lean (total and regional) and fat mass (coefficient of variation (CV) of repeat measures on densitometer: <1.5%). A standard (75 g, 300 mL) two-hour oral glucose tolerance test (OGTT),
with 30 min sampling, was conducted to exclude those participants who may have had type 2 diabetes mellitus (fasting plasma glucose ≥7.0 mmol·L⁻¹ or two-hour plasma glucose ≥11.1 mmol·L⁻¹). At this time, a three-day diet recall was performed. The study diet requirements (described subsequently) were explained and participants were provided a standardised meal (45% carbohydrate, 25% protein and 30% fat; 33% of total daily energy intake (~3700-4300 kJ) based on the Cunningham equation (29)) for consumption the evening prior to a muscle biopsy. Participants were instructed to record their habitual diet for the entire preliminary testing period (~14 days) using a smartphone application (EasyDietDiary (iOS) or MyFitnessPal (Android)). A physical activity monitor (activPAL3 tri-axial accelerometer, PAL-technologies Ltd., Glasgow, Scotland) was worn on the thigh to track habitual physical activity for all of the preliminary testing period, one week of exercise training (i.e. week four) and all of the detraining period. The physical activity monitor was changed weekly. AcitvPAL-derived daily step count and proportions of time spent sitting, standing, stepping and cycling were estimated by exporting data files from the associated proprietary software (PAL Software Suite Version 8.10, PAL-technologies Ltd., Glasgow, Scotland).

Participants reported to the laboratory in an overnight fasted state (~10 h) between 0630-0730 h and underwent measures to estimate resting energy expenditure (REE) (see Document, Supplemental Digital Content 1, Appendix). Immediately following the REE measures, a resting percutaneous muscle biopsy from the vastus lateralis (~100-200 mg) was obtained, under local anaesthesia (2–3 mL of 1% Xylocaine) using a Bergström needle modified for manual suction. One to two pieces of the muscle tissue sample (~30-40 mg per piece) were mounted on a water-soluble compound (Tissue-Tek Optimal Cutting Temperature, Sakura Finetek, Netherlands).
frozen in liquid nitrogen cooled isopentane and stored at -80 °C until analysis. The remaining sample was also frozen in liquid nitrogen and stored at -80 °C until analysis.

Approximately 48 h following a muscle biopsy, the left and right vastus lateralis were scanned by two-dimensional (2D) B-mode ultrasound (frequency, 12 MHz; depth, 8 cm; field of view, 14 x 47 mm) (GE Healthcare Vivid-/, Wauwatosa, WI) to determine muscle thickness from ultrasound images taken along the longitudinal axis of the muscle belly (see Document, Supplemental Digital Content 1, Appendix). After the muscle ultrasound, participants performed a progressive incremental cycling exercise test on a stationary ergometer (Lode, Excalibur sport, Groningen, Netherlands) to determine peak oxygen uptake (VO₂peak) and maximal aerobic power (MAP) (see Document, Supplemental Digital Content 1, Appendix). The MAP achieved at VO₂peak was used to determine exercise training intensities (W) for ENT and HIIT.

Approximately 24-48 h after the VO₂peak test, maximal upper and lower body muscle strength was assessed via a battery of one-repetition maximum (1RM) tests including bilateral leg press (45° incline), bilateral knee extension and bench press. A series of sets (3-5, 2-8 repetitions) at increasing submaximal weights were lifted until the participant reported a rating of perceived exertion (RPE) of ~16 using Borg’s CR6-20 scale. Each 1RM attempt was followed by 5 min of rest. The 1RM for each exercise was determined in isolation (i.e. exercises were not alternated). A maximum of five 1RM attempts were allowed per exercise. The 1RM achieved for each exercise was used to determine RET intensities (%1RM). Following 1RM testing, participants met with the study nutritionist where daily energy (kJ) and protein (g/kg BW⁻¹) intake targets were prescribed for the remainder of the study.
Approximately 48 h following the final exercise training session (i.e., the end of week six), participants reported to the laboratory in an overnight fasted state (~10 h) for post-exercise training testing (Post) in which a body composition scan and vastus lateralis muscle biopsy were performed as described above. After ~48 h, 2D ultrasound images of the left and right vastus lateralis were obtained followed by VO$_2$peak testing. Subsequently (~24-48 h later), 1RM testing was performed. Completion of 1RM strength testing marked the commencement of the detraining period. Two and a half weeks later, post-detraining testing (DT) commenced where measurements were collected in the same order as per Post testing.

Exercise training protocols

All groups performed three morning exercise sessions per week for six weeks. Progressive overload was applied to all exercise training protocols. The primary aim of the HIIT and ENT protocols was to increase aerobic capacity while the overarching goal of the RET protocol was to increase skeletal muscle mass and strength. Participants in HIIT and ENT groups wore a heart rate monitor (Polar H2, Polar, Australia) during each exercise session and RPE was obtained at regular intervals (i.e. at the conclusion of a work period) using Borg’s CR6-20 scale. At the beginning of week four, participants in the HIIT and ENT groups performed a VO$_2$peak test without breath analysis to reassess MAP. At the same time point, individuals in the RET group performed 1RM testing to reassess muscle strength. Based on results from week four exercise testing, training intensities were adjusted accordingly.
Endurance exercise training

A three-minute warm-up (100 W) preceded each training session on a stationary cycle ergometer. Total exercise session duration varied between 30-52 min comprised of 5-8 min work periods at 50-75% peak power output (PPO) with one minute of active rest at 50 W. A three-minute cool-down at 50 W followed the final rest period. The duration and intensity of work periods increased throughout the exercise training program (see Document, Supplemental Digital Content 1, Appendix).

High-intensity interval training

A three-minute warm-up at (100 W) preceded each training session on a stationary cycle ergometer. Total exercise session duration varied between 13-23 min comprised of 30-60 s work periods at 90-130% PPO with one minute of recovery at 50 W. The number of repetitions varied from 8-15 depending on the work period duration for that session. A three-minute cool-down at 50 W followed the final rest period. The duration and intensity of work periods increased throughout the exercise training program (see Document, Supplemental Digital Content 1, Appendix).

Resistance exercise training

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

Dietary intervention and analysis

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

Biochemical and histochemical analyses

Immunohistochemistry

A cryostat (Leica CM1850, Leica Biosystems, Victoria, Australia) was used to obtain serial muscle cross sections (7 μm) which were fixed to specimen slides (SuperFrost Plus, ThermoFisher Scientific, Victoria, Australia), dried at room temperature for 30-60 min and stored at -80°C until analysis. Slides were fixed in 2% formaldehyde (4% formaldehyde solution, Merck & Co, Darmstadt, Germany) for 10 min and washed in phosphate-buffered solution (PBS) for 5 min. The PBS was removed and washed in phosphate-buffered solution with Tween (PBST) for 5 min. The PBST was removed, and the muscle sections were blocked in a solution containing 2% bovine serum albumin (BSA) in PBS, 5% foetal bovine serum, 0.2% Triton X-100, 0.1% sodium azide and 5% goat serum for 90 min. The blocking solution was removed, and sections were incubated in a primary antibody against laminin (rabbit-anti-human, 1:250 [i.e. one-part antibody in 249 parts 1% BSA], ab11575, Abcam, United Kingdom,
RRID:AB_298179) overnight at 4 ºC. The following morning, the primary antibody was removed and slides were washed in PBST (3 x 5 min) and incubated in secondary antibody (goat-anti-rabbit, 1:500, Alexa Fluor 488, Life Technologies, CA, USA, RRID:AB_2633280) for 120 min at room temperature. Sections were then washed in PBST (3 x 5 min), fixed in 2% PFA for 5 min, washed again in PBST (2 x 5 min), blocked with 10% GS in PBS for 90 min and then incubated in a primary antibody against myosin heavy chain slow (MHCI; mouse-anti-human, 1:2, isoform A4.951, DHSB, Iowa, USA, RRID:AB_528385) overnight at 4 ºC. The following morning, sections were washed three times in PBST (3 x 5 min), incubated in secondary antibody (goat-anti-mouse, 1:500, Alexa Fluor 488, Life Technologies, CA, USA, RRID:AB_2633275) for 120 min at room temperature and washed again in PBST (3 x 5 min) before being air dried in the dark for 2 min. One drop of fluorescent mounting medium (ProLong™ Diamond Antifade Mountant, Life Technologies, CA, USA) was then applied to each section and slides were stored at -20 ºC until imaging.

A microscope with a high-resolution fluorescent camera attached was used to view slides. All images were captured through the 20× objective using associated software (EVOS FL Auto 2 cell imaging system, Invitrogen, CA, USA). In this study, 58 ± 8 and 78 ± 16 type I and II muscle fibers, respectively, were counted at each biopsy/participant/time point for CSA. Slides were blinded for both group and time prior to one study researcher (MC) performing all analysis using cell counting software (Fiji, ImageJ Version 2, National Institute of Health, RRID:SCR_002285). The CV between two blinded measurements, completed prior to the commencement of muscle fiber CSA analysis, was 2.7%. Fibers on the periphery of sections were excluded from the analysis. Areas of sections that were affected by freeze fracture artefact
or contained longitudinally oriented fibers were excluded from the analysis. If <50 fibers in total were counted at a time point, all muscle fiber CSA data for that participant at that time point was excluded from the analysis. In this study, 31 participants (ENT: n=11, HIIT: n=10, RET: n=10) were included for muscle fiber CSA analysis.

Blood analyses

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

Statistical analysis

As no previous studies have compared muscle fiber CSA responses between three different types of single-mode exercise training in human skeletal muscle, sample size was determined a priori (G*Power, Version 3.1) using previous literature assessing muscle fiber CSA changes in response to endurance or resistance exercise training (31) with the following inputs: two tailed, effect size (d) = 1.3, α = 0.05 and power = 0.80. Statistical analyses were performed using SPSS software (Version 25, IBM, USA). Data normality was assessed prior to statistical analysis by assessing skewness, kurtosis and results from Shapiro-Wilk tests. Linear mixed effect models (LME), with subject for random intercept, were used to determine main effects of fixed
factors: time (Pre, Post, DT), group (ENT, HIIT, RET), and interaction (time × group) from which residuals were plotted on a histogram to inspect data distribution. For muscle biopsy data, fiber type (i.e., type I and II) was included as a third fixed factor in the LME where muscle fiber CSA was the dependent variable.

Where significant main effects were observed (i.e., time, group or fiber type), post hoc comparisons with Bonferroni correction were used to locate differences. When significant interaction effects were observed, post hoc comparison with Bonferroni corrections was used to determine within and/or between group differences. Where significant group x time interactions were detected, one-way ANOVA tests of group were subsequently performed to compare the change scores (i.e., the difference between pre and post) to identify significant between-group interactions. Significance was accepted at $P<0.05$. All data in text and tables are presented as mean ± standard deviation. All data in figures are presented as mean and individual participant responses. Estimated mean differences with 95% confidence intervals (CI) from the LME are presented in the Supplementary Table 1 (see Table, Supplemental Digital Content 2, Estimated mean differences and 95CIs).

Results

Participant characteristics and dietary intake

At baseline, there were no significant differences in age, body mass index (Table 1), habitual energy or macronutrient intake between groups (Table 1). A significant main effect of time ($P<0.001$) was observed for protein intake relative to body mass. Post-hoc comparisons showed that protein intake relative to body mass increased significantly during exercise training
in all groups \((P<0.001)\) and remained unchanged during the period of detraining. A significant main effect of time \((P=0.007)\) was observed for carbohydrate intake relative to body mass. After detraining, carbohydrate intake relative to body mass decreased significantly compared to baseline in all groups \((P=0.005)\).

**Body composition**

At baseline, there were no significant differences in body mass (BM), total lean mass (LM), appendicular (ALM), leg lean mass (LLM) or body fat percentage between groups (Table 1 and Figure 3). A significant \(time \times group\) interaction effect \((P=0.023)\) was observed for LM. In response to exercise training, LM increased significantly for both RET \((+2.0 \pm 1.0 \text{ kg, } P<0.001)\) and HIIT \((+1.0 \pm 1.2 \text{ kg, } P=0.011)\), but not for ENT. The LM increase from pre to post was greater with RET compared to ENT only \((+1.2 \pm 1.8 \text{ kg, } P=0.041)\). After detraining, LM remained unchanged compared to post-exercise training for both RET and HIIT. After detraining, LM remained significantly elevated compared to baseline for both RET \((+1.0 \pm 1.2 \text{ kg, } P=0.020)\) and HIIT \((+1.0 \pm 1.3 \text{ kg, } P=0.010)\), but not for ENT.

A significant \(time \times group\) interaction effect \((P=0.033)\) was observed for ALM. In response to exercise training, ALM increased significantly for both RET \((+1.3 \pm 1.2 \text{ kg, } P<0.001)\) and HIIT \((+0.8 \pm 0.8 \text{ kg, } P=0.004)\), but not for ENT. After detraining, ALM remained unchanged compared to post-exercise training for both RET and HIIT. After detraining, ALM remained significantly elevated compared to baseline for RET \((+1.0 \pm 1.0 \text{ kg, } P=0.001)\) and HIIT \((+1.0 \pm 0.8 \text{ kg, } P=0.001)\), but not for ENT.
A significant main effect of time \( (P<0.001) \) was observed for LLM, with an increase in LLM in response to exercise training in all groups \( (P<0.001) \), that was maintained after detraining.

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

**1RM muscle strength**

There were no significant differences at baseline for 1RM leg press, leg extension or bench press muscle strength between groups (Figure 4). A significant \( time \times group \) interaction effect \( (P<0.001) \) was observed for 1RM leg press muscle strength (Figure 4A). In response to exercise training, 1RM leg press muscle strength increased significantly in all groups (RET: +70 ± 31 kg, \( P<0.001 \); HIIT: +27 ± 19 kg, \( P<0.001 \); ENT: +16 ± 16 kg, \( P=0.026 \)). The pre-post 1RM leg press muscle strength increase was greater with RET compared to both ENT (+51 ± 38 kg, \( P<0.001 \)) and HIIT (+41 ± 40 kg, \( P=0.001 \)). After detraining, 1RM leg press muscle strength increased significantly compared to post-exercise training for ENT (+16 ± 18 kg, \( P=0.008 \)), but remained unchanged for both RET and HIIT. As such, 1RM leg press between post-training and detraining was higher with ENT compared to both RET (+22 ± 24 kg, \( P=0.003 \)) and HIIT (+17 ± 25 kg, \( P=0.033 \)). Compared to baseline, 1RM leg press muscle strength remained significantly elevated after detraining (all \( P<0.001 \)).

A significant \( time \times group \) interaction effect \( (P=0.022) \) was observed for 1RM leg
extension muscle strength (Figure 4B). In response to exercise training, 1RM leg extension muscle strength increased significantly for both RET (+23 ± 11 kg, \( P<0.001 \)) and HIIT (+11 ± 11 kg, \( P=0.005 \)) while there was a trend for and increase for ENT (+8 ± 12 kg, \( P=0.064 \)). The increase in 1RM leg extension muscle strength between rest and post-training with RET was only higher than ENT (+14 ± 19 kg, \( P=0.024 \)). After detraining, 1RM leg extension muscle strength remained unchanged in all groups compared to post-exercise training. After detraining, 1RM leg extension muscle strength remained significantly elevated compared to baseline (all \( P<0.05 \)).

A significant time × group interaction effect (\( P=0.001 \)) was observed for 1RM bench press muscle strength (Figure 4C). In response to exercise training, 1RM bench press muscle strength increased significantly for both RET (+10 ± 4 kg, \( P<0.001 \)) and HIIT (+5 ± 6 kg, \( P=0.034 \)), but not for ENT, with this increase greater between RET and ENT (+7 ± 8 kg, \( P=0.005 \)). After detraining, 1RM bench press muscle strength remained unchanged compared to post-exercise training for both RET and HIIT. After detraining, 1RM bench press muscle strength remained significantly elevated compared to baseline for RET (+15 ± 13 kg, \( P<0.001 \)), but not for HIIT or ENT.

A significant time × group interaction effect (\( P=0.001 \)) was observed for the sum of all 1RM (Figure 4D). In response to exercise training, the sum of all 1RM increased significantly in all groups (RET: +90 ± 38 kg, \( P<0.001 \); HIIT: +41 ± 23 kg, \( P=0.001 \); ENT: +23 ± 21 kg, \( P=0.002 \)). The increase in the sum of 1RM was greater with RET compared to both ENT (+71 ± 42 kg, \( P<0.001 \)) and HIIT (+53 ± 43 kg, \( P<0.001 \)). After detraining, the sum of all 1RM
remained unchanged compared to post-exercise training in all groups. After detraining, the sum of all 1RM s remained significantly elevated compared to baseline (all $P<0.05$).

**VO$_2$peak and maximal aerobic power**

At baseline, there were no significant differences in VO$_2$peak or maximal aerobic power (MAP) between groups (Figure 5). A significant $time \times group$ interaction effect ($P=0.001$) was observed for VO$_2$peak (Figure 5A). In response to exercise training, VO$_2$peak increased significantly for both HIIT ($+0.4 \pm 0.2$ L min$^{-1}$, $14 \pm 7\%$, $P<0.001$) and ENT ($+0.3 \pm 0.3$ L min$^{-1}$, $11 \pm 11\%$, $P<0.001$), but not for RET. Indeed, the increase in VO$_2$peak post-training was higher with HIIT compared to RET ($+0.3 \pm 0.3$ L min$^{-1}$, $P=0.003$). After detraining, VO$_2$peak decreased significantly compared to post-exercise training for HIIT ($-0.2 \pm 0.1$ L min$^{-1}$, $-6 \pm 4\%$, $P=0.005$) and there was a trend for a decreased VO$_2$peak for ENT ($-0.1 \pm 0.2$ L min$^{-1}$, $-4 \pm 5\%$, $P=0.055$). After detraining, VO$_2$peak remained elevated compared to baseline for both HIIT ($+0.2 \pm 0.2$ L min$^{-1}$, $8 \pm 6\%$, $P=0.001$) and ENT ($+0.2 \pm 0.2$ L min$^{-1}$, $6 \pm 7\%$, $P=0.009$), but not for RET. As such, VO$_2$peak was higher between baseline and detraining after HIIT compared to RET ($+0.2 \pm 0.4$ L min$^{-1}$, $P=0.043$).

A significant $time \times group$ interaction effect ($P<0.001$) was observed for MAP (Figure 5B). In response to exercise training, MAP increased significantly for both HIIT ($+30 \pm 9$ W, $P<0.001$) and ENT ($+26 \pm 14$ W, $P<0.001$), but not for RET. The change in MAP between rest and post-training was higher with ENT compared to RET ($+16 \pm 20$ W, $P=0.007$) as well as with HIIT compared to RET ($+20 \pm 20$ W, $P=0.001$). After detraining, MAP decreased significantly for HIIT ($-17 \pm 11$ W, $P=0.001$) compared to post-exercise training but remained unchanged for
ENT. The decrease in MAP with HIIT from post-exercise to detraining was significantly greater compared to ENT (-13 ± 15 W, P=0.004) and RET (-13 ± 16 W, P=0.007). After detraining, MAP remained significantly elevated compared to baseline for both HIIT (+13 ± 14 W, P<0.001) and ENT (+23 ± 13 W, P<0.001), but not for RET. MAP was significantly higher from baseline to detraining between HIIT and RET only (+17 ± 21 W, P=0.009).

**Muscle fiber characteristics**

At baseline, there were no significant differences in type I or II muscle fiber cross-sectional area (CSA) between groups (Table 2). There was no significant time × group × fiber type interaction effect for muscle fiber CSA. A significant main effect of time was observed for type I and II muscle fiber CSA in all groups (all, P=0.007). In response to exercise training, type I and type II muscle fiber CSA increased significantly (P=0.006) in all groups. After detraining, type I and II muscle fiber CSA remained unchanged compared to post-exercise training in all groups.

At baseline, there were no significant differences in the proportion of type I or type II fibers between groups (Table 3). A significant main effect of time was observed for both type I and II fiber type percentages (both P<0.05), however, no significant differences between time points could be detected in all groups.

**Muscle thickness**

At baseline, there were no significant differences in muscle thickness (MT) between groups (Table 1). A significant time × group interaction effect (P<0.001) was observed for
muscle thickness (Table 1). In response to exercise training, MT increased significantly for both RET (+0.3 ± 0.2 cm, P<0.001) and HIIT (+0.3 ± 0.2 cm, P<0.001), but not for ENT. As such, changes in MT between baseline and post-training were greater between RET and ENT (+0.3 ± 0.2 cm, P<0.001) and between HIIT and ENT (+0.3 ± 0.2 cm, P<0.001). After detraining, MT decreased significantly compared to post-exercise training for RET (-0.3 ± 0.1 cm, P<0.001), but remained unchanged for HIIT. Changes between post-training and detraining were greater with RET compared to ENT (+0.3 ± 0.2 cm, P=0.000) as well as with HIIT compared RET (+0.2 ± 0.2 cm, P=0.015). After detraining, MT remained significantly elevated compared to baseline for HIIT (+0.2 ± 0.2 cm, P<0.001), but returned to pre-exercise training levels for RET.

Resting energy expenditure & oral glucose tolerance test

At baseline, there were no significant differences in REE between groups (Table 3). REE did not change after exercise training or detraining in all groups.

At baseline, there were no differences between groups for any measurements derived from the OGTT (Table 3). A significant time × group interaction effect (P=0.001) was observed for fasting plasma glucose. In response to exercise training, fasting plasma glucose increased significantly for RET (+0.4 ± 0.6 mmolL⁻¹, P=0.001), but not HIIT or ENT. After detraining, fasting plasma glucose decreased significantly compared to post-exercise training for RET (-0.4 ± 0.5 mmolL⁻¹, P=0.002) that was also greater compared to ENT (+0.5 ± 0.7 mmolL⁻¹, P=0.049). Total glucose area under the curve did not change after exercise training or detraining in all groups.
A significant main effect of *time* ($P=0.035$) was observed for fasting plasma insulin. Post-hoc comparisons showed a significant decrease for fasting plasma insulin after detraining ($P=0.038$) compared to baseline. A trend for a *time* × *group* interaction effect was observed for total insulin area under the curve ($P=0.061$). HOMA2-IR did not change after exercise training or detraining in all groups.

**Physical activity**

At baseline, there were no significant differences in physical activity measurements between groups (see Table, Supplemental Digital Content 3, Physical activity). A trend for a main effect of *time* was observed for daily step count ($P=0.067$). A significant main effect of *time* was observed for the percentage of the day spent moving ($P=0.011$) and standing ($P=0.023$). Post-hoc comparisons showed a reduction in the percentage of the day spent moving ($P=0.011$) and standing ($P=0.020$) during detraining compared to week four of exercise training.

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

A significant *time* × *group* interaction effect ($P<0.001$) was observed for the percentage of the day spent cycling. At week four of exercise training, the percentage of the day spent cycling increased significantly compared to baseline for ENT (+1 ± 1%, $P<0.001$), but not for HIIT or RET. At week four of exercise training, the percentage of the day spent cycling was significantly greater for ENT compared to HIIT (+1 ± 1%, $P=0.010$) and RET (+2 ± 1%, $P<0.001$).
difference at week four in the percentage of the day spent cycling was greater with ENT compared to RET (+1 ± 2%, P=0.003).

Discussion

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

Exercise training responses

Muscle strength & aerobic capacity

Physical fitness prior to surgery is an independent predictor of post-operative morbidity and mortality (6). Pre-operative exercise training is one intervention that can enhance physical fitness and better prepare an individual for subsequent surgery. However, pre-operative exercise training programming require optimized prescription (e.g., single- vs dual-mode) to meet the individual needs of various clinical populations within a short time-frame (4). After six weeks of exercise training we observed that all exercise modalities increased whole-body muscle strength (i.e. sum of all 1RM$s$), although exercise training-induced increases in aerobic capacity were only induced after HIIT and ENT. To the best of our knowledge, only one other study has directly compared the effects of ENT, HIIT and RET on muscle strength and aerobic capacity in middle-age adults (32). In that investigation, 12 weeks of RET increased 1RM leg press muscle strength (+25%) but walking/running HIIT and ENT did not (32). In contrast, we observed an
increase in 1RM leg press muscle strength with cycling HIIT (+11%) and ENT (+8%), although these changes were less than the training-induced increase observed with RET. While it is difficult to explain why our shorter HIIT and ENT protocols (i.e., six vs 12 weeks) increased 1RM muscle strength, key differences were apparent in study designs between our current work and that of Schjerve and co-workers (32). Firstly, our exercise program involved a progressive overload where we retested all exercise groups halfway through our training intervention to readjust training zones to increasing strength adaptations. Secondly, we increased dietary protein intake (~1.4 g·kg·BW·d⁻¹) to help augment anabolic adaptations to exercise training. Finally, all our training sessions were supervised in the laboratory to monitor first-hand training technique and ensure appropriate training intensity. Collectively, increases in muscle strength with short-term aerobic-based exercise training may be dependent on incorporating supervised training programs that provide appropriate training intensity/progressive overload and with supportive nutritional measures. However, an important limitation to this inference is that our study only recruited males. Whether females could similarly increase muscle strength with short-term HIIT, particularly in light of substantial differences in hormonal milieu between sexes in middle-age adulthood, remains an area of further investigation. Additionally, we cannot discount the potential of a ‘learned effect’ that may explain our current strength results. Thus, future studies that incorporate appropriate strength testing familiarisation sessions preceding short-term HIIT and END training are warranted. We also found that aerobic capacity increased with HIIT and ENT (+14% and +11%, respectively), but not after RET, in agreement with previous reports in middle-age adults (9, 33-36). Increased aerobic capacity of a similar magnitude (~10%) to that seen after HIIT and ENT have been reported following 12 weeks of RET in middle-age adults (32). Therefore, the RET programme used in the current study may not be the most suitable
where short-term RET-induced improvements in aerobic capacity are desired. Alternatively, circuit-based RET involving whole-body movement that induces greater stress on the cardiovascular system, while also increasing muscle strength, may provide a better option for enhancement of both components of physical function. Although our participants were not pre-operative patients, our findings demonstrate that short-term HIIT and ENT can lead to improved muscle strength following short-term exercise training. Short-term cycling-based exercise training (e.g. ENT or HIIT) may be of use in clinical scenarios where the surgery patient cannot or does not wish to participate in strength-based exercise training (e.g. physical restrictions, access to equipment, instructor availability).

Lean mass, muscle thickness and muscle fibre size

In many populations, reduced functional capacity and skeletal muscle mass is a predictor of unfavourable post-operative outcomes (15, 16, 37). Thus, pre-operative exercise training-induced increases in skeletal muscle mass can better prepare the patient for surgery and the ensuing recovery period (7). There is a paucity of information regarding lean mass responses following different types of short-term exercise training in both pre-surgery patients and healthy middle-age adults. In the current study, the largest increase in lean mass was induced by RET (+2 kg) although HIIT also significantly increased lean mass (+1 kg), while there was no change observed after ENT. While this increase in lean mass with HIIT was statistically significant, it was similar to the CV of the densitometer used to obtain lean mass measurements (i.e., 1.6 vs 1.5%) and should be interpreted with caution. Robinson and co-workers (38) reported increases in fat-free mass (FFM) in young and older adults following 12 weeks of RET or HIIT (~2 kg and ~1 kg, respectively). Despite our shorter exercise training protocols, we observed similar
changes in FFM (data not shown) after RET and HIIT. Unlike our study, Robinson and co-workers (38) did not control for dietary protein intake. We ensured participants met a protein target (~1.4 g kg BW$^{-1}$ d$^{-1}$) recommended to promote and maintain muscle growth while exercise training (30) as part of a free-living eating plan that may have augmented gains in lean mass. Without a non-protein supplemented exercise training group (e.g. ~1.0 g kg BW$^{-1}$ d$^{-1}$) we cannot evaluate the contribution of increased protein intake to observed gains in lean mass.

Six weeks of RET and HIIT but not ENT increased *vastus lateralis* muscle thickness (+11% and 10%, respectively). We have previously reported that 12 weeks of RET and ENT increased *vastus lateralis* muscle thickness (+14% and 10%, respectively) in young recreationally active men consuming a high-protein diet (2 g kg BW$^{-1}$ d$^{-1}$) (39). Notably, ENT comprised interval-style exercise sessions in the final month of the programme in that study (39). Our results show that improvements of a similar magnitude are attainable after short-term RET and HIIT in middle-age men. However, as ultrasound measures occurred ~24-48 hours following muscle biopsies in all participants due to unavoidable logistical reasons, we cannot rule out any possible effects of local oedema/swelling in the *vastus lateralis* that may have affected muscle thickness measurements. Taken together, these findings suggest that higher-intensity intermittent exercise (aerobic- or resistance-based) may be an important consideration where exercise training-induced increases in muscle mass/thickness are required in a relatively short period of time (i.e. six weeks) in middle-age men. To the best of our knowledge, this is the first study to report changes in muscle fiber size following ENT, HIIT and RET in middle-age adults. De Souza and co-workers (40) observed muscle fiber hypertrophy (type I and IIa) following eight weeks of RET but not HIIT in young men. As the HIIT intervention in that study involved
treadmill running, it is plausible the high eccentric component of this contractile mode may have induced greater muscle damage and thus limited its anabolic potential. Indeed, previous work has reported running-based activity to produce greater attenuation of lower-body muscle strength and hypertrophy compared with cycling (41). Farup and co-workers (31) reported increased type II but not type I muscle fiber size in response to ten weeks of RET but not ENT (that included one weekly HIIT session) in young, untrained men. While these findings are difficult to reconcile considering the similarities in participant training status and mode of ENT, the potential for aerobic-based exercise to increase muscle fiber CSA is an area of research requiring further investigation that may confer health and performance benefits. We must also acknowledge that our small sample size for CSA analyses and the variation in resting fiber CSA within our sedentary middle-age participants which may have limited the potential to detect post-training and detraining CSA differences.

The current findings demonstrate that short-term HIIT can concurrently increase aerobic capacity and lean mass, an outcome that would typically be achieved using combined exercise training. As HIIT is a time-efficient intervention and may be perceived as more enjoyable to undertake compared to ENT (42), HIIT may be appealing to some pre-surgery patients. However, future studies comparing short-term HIIT and combined exercise training in surgery patients are needed to confirm if HIIT can match the beneficial physical effects of combined exercise training.

Resting energy expenditure & glucose homeostasis

Fat-free mass, including metabolically active tissue such as skeletal muscle, is a large
determinant of resting energy expenditure (REE) (43). Increases in surrogates of muscle mass (e.g. lean/fat-free mass) and REE have been reported following different types of prolonged exercise training in some (44) but not all studies (45, 46). In the current study, both RET and HIIT increased lean mass without detectable changes in REE. Exercise training did not improve fasting glucose, insulin or respective areas under the curve following a two-hour OGTT, most likely because our participants had good glycaemic control prior to the study.

**Detraining responses**

*Muscle strength & aerobic capacity*

Short periods (~two weeks) of reduced physical activity induce skeletal muscle deconditioning (47, 48). Exercise training can attenuate catabolic events typically observed during periods of reduced physical activity (49). However, studies addressing the exercise modality that best preserves physical fitness and skeletal muscle adaptation responses following short-term exercise training cessation are few. We observed exercise training-induced gains in muscle strength were maintained following 2.5 weeks of detraining, despite declines in aerobic capacity. Although our participants were ambulatory during the detraining period, we observed a reduction in the percentage of the day spent moving and an increase in percentage of the day spent sitting (main effects of time).

Spence and co-workers (50) investigated the effects of six weeks of detraining following six months of ENT or RET on aerobic capacity and muscle strength in young men. Detraining resulted in a significant decline in aerobic capacity with ENT despite the maintenance of lower body muscle strength (1RM squat). In contrast, upper (1RM bench press) and lower body muscle
strength gains persisted after detraining following the RET intervention (50). In the current study, we observed similar patterns for changes in aerobic capacity and muscle strength following detraining with ENT and RET. Declines in VO₂peak concomitant with loss of capillarisation and mitochondrial enzyme activities have been reported following 2-4 weeks of detraining proceeding short-term aerobic exercise training (51, 52). Additionally, a loss of plasma volume may have also contributed to observed decreases in aerobic capacity following short-term detraining (53). Overall, our results and previous work (50) indicate that short-term and prolonged ENT-induced increases in aerobic capacity are lost rapidly (~2-6 weeks) compared to the time-course for declines in muscle strength in young and middle-age men.

Lean mass, muscle thickness and muscle fibre size

We found exercise training-induced increases in surrogates of whole-body and regional muscle mass after RET and HIIT were maintained following a short period of exercise training cessation similar in duration to the early post-operative period faced by patients who have undergone surgery. As such, short-term single-mode higher-intensity exercise training prior to periods of forced inactivity may provide benefits to physical function, combatting the catabolic effects of reduced physical activity typically observed following inactivity and/or surgery (54, 55). However, it should also be noted that leg press muscle strength gains were preserved following detraining with ENT. As lower body muscle strength contributes to mobility, we speculate that different types of single-mode exercise training (albeit to varying magnitudes) are capable of retaining components important for physical function. Whether preservation of lower body muscle strength aligns with improved early post-operative clinical outcomes (e.g. less time in hospital, reduced post-operative complications) in surgery cohorts following different types of
short-term exercise training remains to be determined. In the current study, type I and II muscle fiber CSA was maintained after detraining independent of exercise training modality. However, we must acknowledge that both type I and II fiber CSA were numerically lower than post-exercise training levels after detraining in the RET (type I: -11%, type II: -16%) and HIIT groups (type I: -20%, type II: -10%). While this reduction was not statistically significant likely due to low sample size, it cannot be discounted that the observed decrease with detraining represents a clinically relevant decrease in muscle fiber size.

Although the middle-age men in the current study are likely to have been more physically active during the detraining period than many post-operative patients, early ambulation is often encouraged where possible following surgery (2). Thus, we believe our results provide proof-of-principle that short-term single-mode higher intensity exercise training can counter some of the catabolic effects in skeletal muscle induced by short-term reduced physical activity (e.g. decreased muscle mass). Whether single-mode exercise training can benefit muscle adaptation responses shortly before and after surgery needs to be explored in a variety of surgery populations (e.g. elective surgery, oncology).

Conclusion

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

Figure 1. Participant recruitment flow.

Figure 2. Study design. Overview of when measurements were taken throughout the study. Exercise training intensities were adjusted where necessary at week 4. All measurements were obtained under resting conditions except for the post-exercise training muscle biopsy collected ~48 h following the final exercise training session (end of week 6). Post-exercise training testing (Post) took place across study weeks 6 and 7 and post-detraining testing (DT) across study weeks 9 and 10.

Figure 3. Baseline (Pre) total (A), appendicular (B) and leg (C) lean mass and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-age men.
Data are presented as mean and individual responses. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. a, $P<0.05$ vs Pre within group; b, $P<0.05$ vs Post within group; *, main effect of time ($P<0.05$) vs Pre. RET at DT: n=10.

Figure 4. Baseline (Pre) leg press 1RM (A), leg extension 1RM (B), bench press 1RM (C), sum of all 1RMs (D) and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-age men.
Data are presented as mean and individual responses. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. 1RM, one-repetition
maximum. a, P<0.05 vs Pre within group; b, P<0.05 vs Post within group. ENT, bench press: n=11; HIIT, leg press at Post: n=11; RET at DT: n=10.

Figure 5. Baseline (Pre) VO₂peak (A), maximal aerobic power (B) and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-age men. Data are presented as mean and individual responses. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. W, watt. a, P<0.05 vs Pre within group; b, P<0.05 vs Post within group. HIIT at DT: n=11; RET at DT: n=10.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Appendix

SDC 2: Supplementary Table S1. Estimated mean differences and 95CIs.docx

SDC 3: Supplementary Table S2. Physical activity.docx
Figure 2

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Figure 3

(A) Total lean mass (kg)

(B) Appendicular lean mass (kg)

(C) Leg lean mass (kg)
Figure 4
Figure 5

A

VO\textsubscript{peak} (L/min\textsuperscript{-1})

B

Maximal aerobic power (W)
## Table 1. Baseline participant characteristics and macronutrient intake (Pre) and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

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<td>28.0 ± 3.0</td>
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<td>27.4 ± 2.7</td>
</tr>
<tr>
<td>Body fat %</td>
<td>31.8 ± 6.1</td>
<td>31.4 ± 6.1</td>
<td>31.3 ± 6.4</td>
<td>30.8 ± 3.7</td>
</tr>
<tr>
<td>Muscle thickness (cm)</td>
<td>2.6 ± 0.3</td>
<td>2.6 ± 0.4</td>
<td>2.6 ± 0.3</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Energy (kJ·d⁻¹)</td>
<td>9707 ± 1649</td>
<td>1000 ± 1047</td>
<td>9532 ± 1555</td>
<td>10680 ± 2658</td>
</tr>
<tr>
<td>Protein (g·kg BW⁻¹)</td>
<td>1.3 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>CHO (g·kg BW⁻¹)</td>
<td>2.4 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.2 ± 0.6</td>
<td>2.9 ± 1.0</td>
</tr>
<tr>
<td>Fat (g·kg BW⁻¹)</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

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

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Table 2. Baseline *vastus lateralis* muscle fiber characteristics (Pre) and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

<table>
<thead>
<tr>
<th></th>
<th>ENT</th>
<th>HIIT</th>
<th>RET</th>
<th>Main effects (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I CSA (µm²)</td>
<td>Pre 3672 ± 776</td>
<td>Post 4245 ± 1309</td>
<td>DT 4268 ± 1336</td>
<td>Time 0.007</td>
</tr>
<tr>
<td>Type II CSA (µm²)</td>
<td>Pre 4269 ± 943</td>
<td>Post 4949 ± 2060</td>
<td>DT 4804 ± 1548</td>
<td>Time 0.007</td>
</tr>
<tr>
<td>Type I %</td>
<td>Pre 46 ± 12</td>
<td>Post 41 ± 10</td>
<td>DT 49 ± 14</td>
<td>Time 0.034</td>
</tr>
<tr>
<td>Type II %</td>
<td>Pre 54 ± 12</td>
<td>Post 59 ± 10</td>
<td>DT 51 ± 14</td>
<td>Time 0.034</td>
</tr>
</tbody>
</table>

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. CSA, muscle fiber cross-sectional area. Fiber type % is ENT: n=11; HIIT: n=10; RET: n=10.
Table 3. Baseline (Pre) resting energy expenditure and oral glucose tolerance measurements and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

<table>
<thead>
<tr>
<th></th>
<th>ENT</th>
<th>HIIT</th>
<th>RET</th>
<th>Main effects (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting metabolic rate test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting energy expenditure (kcal)</td>
<td>1974 ± 710</td>
<td>1968 ± 210</td>
<td>1926 ± 198</td>
<td>0.349</td>
</tr>
<tr>
<td></td>
<td>1955 ± 210</td>
<td>1941 ± 210</td>
<td>1942 ± 204</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1955 ± 210</td>
<td>2056 ± 210</td>
<td>2014 ± 210</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1922 ± 198</td>
<td>2028 ± 190</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.486</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.924</td>
</tr>
<tr>
<td><strong>Oral glucose tolerance test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol L⁻¹)</td>
<td>5.4 ± 0.5</td>
<td>5.2 ± 0.5</td>
<td>5.1 ± 0.3</td>
<td>0.848</td>
</tr>
<tr>
<td></td>
<td>5.1 ± 0.3</td>
<td>5.2 ± 0.9</td>
<td>5.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.1 ± 0.5</td>
<td>5.1 ± 0.9</td>
<td>5.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.1 ± 0.9</td>
<td>0.501</td>
<td>0.001</td>
<td></td>
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<tr>
<td>AUCtotal glucose (mmol h⁻¹ L⁻¹)</td>
<td>893 ± 193</td>
<td>824 ± 131</td>
<td>899 ± 179</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>726 ± 99</td>
<td>721 ± 108</td>
<td>731 ± 154</td>
<td></td>
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<tr>
<td></td>
<td>874 ± 165</td>
<td>798 ± 139</td>
<td>773 ± 146</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7056 ± 210</td>
<td>7671 ± 139</td>
<td>7239 ± 4819</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>7239 ± 146</td>
<td>0.113</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma insulin (mIU L⁻¹)</td>
<td>7.4 ± 5.9</td>
<td>6.0 ± 5.1</td>
<td>6.7 ± 5.4</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>6.7 ± 2.7</td>
<td>5.5 ± 2.8</td>
<td>4.5 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.8 ± 7.7</td>
<td>8.1 ± 6.6</td>
<td>7.9 ± 4.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7239 ± 146</td>
<td>0.246</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCtotal insulin (mmol h⁻¹ L⁻¹)</td>
<td>6098 ± 3810</td>
<td>6096 ± 6496</td>
<td>7136 ± 6297</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>4563 ± 2063</td>
<td>3955 ± 1883</td>
<td>4194 ± 2118</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3955 ± 1883</td>
<td>9277 ± 5751</td>
<td>7271 ± 4196</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>7239 ± 4819</td>
<td>7239 ± 4819</td>
<td>7239 ± 4819</td>
<td>0.061</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>1.0 ± 0.7</td>
<td>0.8 ± 0.7</td>
<td>0.9 ± 0.7</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.4</td>
<td>0.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2 ± 1.0</td>
<td>1.1 ± 0.9</td>
<td>1.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.1 ± 0.6</td>
<td>0.207</td>
<td>0.559</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. AUC, area under the curve; HOMA2-IR, homeostatic model assessment of insulin resistance. a, P<0.05 vs Pre within group; b, P<0.05 vs Post within group. RET at DT: n=10.
Title: Skeletal muscle adaptive responses to different types of short-term exercise training and detraining in middle-aged men

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Swinburne University of Technology,
Supplementary Material

1. Supplementary Methods

Participant allocation to training group

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

Blood sampling protocol

Fasted blood glucose was determined using a handheld glucometer (Accu-Chek Performa II, Roche Diagnostics Ltd., Basel, Switzerland). If blood glucose was <6.9 mmol L⁻¹ (i.e. absence of type 2 diabetes), participants consumed a glucose solution containing 75 g of glucose (PoC Diagnostics, North Rocks, NSW, Australia). Blood glucose was again determined using the
handheld glucometer immediately following collection of the final OGTT sample. Participation was confirmed if the sample concentration was <11.0 mmol·L⁻¹ (i.e. absence of glucose intolerance). Blood samples were centrifuged at 4 °C for 10 min at 4,095 rpm and plasma aliquots were stored at -80 °C until analysis.

*Resting energy expenditure test protocol*

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

*Muscle ultrasound assessment*

Muscle thickness of the left and right vastus lateralis were determined from ultrasound images taken along the longitudinal axis of the muscle belly utilizing a two-dimensional, B-mode ultrasound (frequency, 12 MHz; depth, 8 cm; field of view, 14 x 47 mm) (GE Healthcare Vivid-i, Wauwatosa, U.S.A). Images were taken at 50% (mid) of the distance between the central palpable point of the greater trochanter and the lateral condyle of the femur. Once the scanning site was determined, the distances from various anatomical landmarks were recorded to ensure reproducibility for future testing sessions. These landmarks included the ischial tuberosity, fibula head and the greater trochanter. On subsequent visits the scanning sites were determined and marked on the skin and then confirmed by replicated landmark distance measures. All architectural assessments were performed with participants in a supine position with the hip and
knee in a neutral position following at least 5 min of inactivity. To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning sites, aligned parallel to the muscle fascicles and perpendicular to the skin. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence measurement accuracy (1). Finally, the probe orientation was manipulated slightly by the assessor (RGT) if the superficial and deep aponeuroses were not parallel.

Once the images were collected, analysis was undertaken off-line (MicroDicom, Version 0.7.8, Bulgaria). At each site muscle thickness was defined as the distance between the superficial and deep aponeuroses of the vastus lateralis. The superficial and deep aponeurosis angles were determined as the angle between the line marked as the aponeurosis and an intersecting horizontal reference line across the captured image (2, 3).

The same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers (name and group) during the collection and analysis of the images. The assessor is reliable with intraclass correlations (ICCs) for muscle thickness ranging from 0.97 to 0.99, typical error (TE) from 0.09 to 0.22 cm, typical error as a percentage (%TE) from 1.0 to 3.9% and a minimum detectable change (MDC) from 0.25 to 0.61 cm.

Cycling VO2peak and maximal aerobic power test protocol

Participants were fitted with a heart rate monitor and performed a 5-minute warm-up at a power output of one watt per kilogram of body weight (Wkg⁻¹) whilst wearing a mouthpiece (Hans Rudolf Inc., Kansas, USA) for collection of expired breath connected to a calibrated (O₂: 16%, CO₂: 4%) metabolic cart (TrueOne 2400, ParvoMedics, Utah, USA). During the test, power output increased by 25 W every 2.5 min (4). Within the final 15 sec of each stage,
participants were asked for their rating of perceived exertion (RPE) using Borg’s CR6-20 scale (5). Participants were required to maintain a cadence >70 rpm until volitional exhaustion.

IRM muscle strength testing movement protocols

Participants were shown correct technique for each exercise by a study researcher prior to any repetitions being performed. No weight (kg) was applied for the participant’s first warm-up set to familiarize them with the verbal cues to distinguish a successful lift and safety mechanisms in place if the attempted weight was too heavy. For the 45º incline bilateral leg press, the 1RM attempt was deemed successful if the participant initiated the lift in ~0º knee extension, lowered the sled to ~90º knee flexion and returned the sled to ~0º knee extension. For the bilateral knee extension, the 1RM attempt was deemed successful if the participant initiated the lift in ~90º knee flexion and lifted the weight to ~0º knee extension. For the bench press, the 1RM attempt was deemed successful if the participant initiated the lift in ~0º elbow flexion, lowered the weighted barbell until touching the chest for approximately one second and returned the weighted barbell to ~0º elbow flexion.
2. Supplementary Results

*Relative 1RM muscle strength*

At baseline, there were no significant differences in 1RM leg press, leg extension or bench press muscle strength between groups. A significant *time × group* interaction effect (*P*<0.001) was observed for change in relative 1RM leg press muscle strength (Figure S1A). In response to exercise training, relative 1RM leg press muscle strength increased significantly in all groups (RET: +0.7 ± 0.3 kg·kg BM⁻¹, *P*<0.001; HIIT: +0.3 ± 0.2 kg·kg BM⁻¹, *P*=0.001; ENT: +0.2 ± 0.2 kg·kg BM⁻¹, *P*=0.028). After detraining, relative 1RM leg press muscle strength increased significantly for ENT compared to post-training (+0.2 ± 0.2 kg·kg BM⁻¹, *P*=0.013), but HIIT and RET remained unchanged. After detraining, relative 1RM leg press muscle strength remained elevated compared to baseline in all groups (RET: +0.6 ± 0.3 kg·kg BM⁻¹, *P*<0.001; HIIT: +0.3 ± 0.3 kg·kg BM⁻¹, *P*<0.001; ENT: +0.4 ± 0.3 kg·kg BM⁻¹, *P*<0.001).

A significant main effect of *time* (*P*<0.001) was observed for change in relative 1RM leg extension muscle strength (Figure S1B). Post-hoc pairwise comparisons showed that 1RM leg extension muscle strength increased significantly after exercise training in all groups (*P*<0.001) and remained elevated after detraining.

A significant *time × group* interaction effect (*P*=0.001) was observed for change in relative 1RM bench press muscle strength (Figure S1C). In response to exercise training, relative 1RM bench press muscle strength increased significantly for RET (+0.10 ± 0.03 kg·kg BM⁻¹, *P*=0.001) and there was a trend for an increase for HIIT (*P*=0.057). After detraining, relative 1RM bench press muscle strength remained unchanged compared to post-exercise training for RET. After detraining, relative 1RM bench press muscle strength remained elevated compared to baseline for RET (+0.15 ± 0.13 kg·kg BM⁻¹, *P*<0.001), but not for HIIT or ENT.
Figure S1. Baseline (Pre) leg press (A), leg extension (B) and bench press (C) relative to body mass 1RM and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

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

Relative VO\textsubscript{2}peak and relative maximal aerobic power

At baseline, there were no significant differences in relative VO\textsubscript{2}peak or relative maximal aerobic power (MAP). A significant time × group interaction effect (P=0.002) was observed for change in relative VO\textsubscript{2}peak (Figure S2A). In response to exercise training, relative VO\textsubscript{2}peak increased significantly for HIIT (+4.1 ± 2.7 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, P<0.001) and ENT group (+3.5 ± 2.9 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, P<0.001), but not for RET. After detraining, relative VO\textsubscript{2}peak decreased compared to post-exercise training for HIIT (-1.9 ± 1.7 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, P = 0.029) and ENT (-1.7 ± 1.7 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, P=0.024). After detraining, relative VO\textsubscript{2}peak remained elevated compared to baseline for HIIT (+2.3 ± 2.8 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, P=0.024) and ENT (+1.8 ± 1.9 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, P=0.003), but not for RET.

A significant time × group interaction effect (P<0.001) was observed for change in relative MAP (Figure S2B). In response to exercise training, relative MAP increased
significantly for HIIT (+0.4 ± 0.2 W·kg\(^{-1}\), \(P<0.001\)) and ENT (+0.3 ± 0.2 W·kg\(^{-1}\), \(P<0.001\)), but not for RET. After detraining, relative MAP decreased significantly compared to post-exercise training for HIIT (-0.2 ± 0.2 W·kg\(^{-1}\), \(P=0.001\)), but remained unchanged for ENT. After detraining, relative MAP remained elevated compared to baseline for both ENT (+0.2 ± 0.2 W·kg\(^{-1}\), \(P<0.001\)) and HIIT (+0.1 ± 0.2 W·kg\(^{-1}\), \(P=0.034\)), but not for RET.
Figure S2. Baseline (Pre) VO₂peak relative to body mass (A) and maximal aerobic power relative to body mass (B) and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.
Data are presented as mean and individual responses. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training; W, watt.  

\(^a\), \(P<0.05\) vs Pre within group;  

\(^b\), \(P<0.05\) vs Post within group. HIIT at DT: n=11; RET at DT: n=10.
3. References


Table S1. Estimated mean differences within intervention groups with 95% confidence intervals.

<table>
<thead>
<tr>
<th></th>
<th>ENT</th>
<th>HIIT</th>
<th>RET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ Pre-Post</td>
<td>Δ Post-DT</td>
<td>Δ Pre-Post</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>-0.1 (-1.8, 1.9)</td>
<td>-0.1 (-1.7, 2.0)</td>
<td>0.2 (-1.7, 2.1)</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>0.0 (-0.4, 0.4)</td>
<td>0.0 (-0.5, 0.4)</td>
<td>0.0 (-0.4, 0.5)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.4 (-0.3, 1.2)</td>
<td>-0.1 (-0.8, 0.7)</td>
<td>0.4 (-0.4, 1.2)</td>
</tr>
<tr>
<td>Leg lean mass (kg)</td>
<td>0.3 (-0.3, 0.8)</td>
<td>0.1 (-0.5, 0.6)</td>
<td>0.3 (-0.2, 0.9)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-0.5 (-1.4, 0.4)</td>
<td>-0.01 (-1.0, 0.8)</td>
<td>-0.5 (-1.4, 0.3)</td>
</tr>
<tr>
<td>Type I CSA (μm²)</td>
<td>462 (-399, 1322)</td>
<td>46 (-791, 882)</td>
<td>507 (-329, 1343)</td>
</tr>
<tr>
<td>Type II CSA (μm²)</td>
<td>569 (-292, 1429)</td>
<td>-122 (-958, 714)</td>
<td>447 (-389, 1283)</td>
</tr>
<tr>
<td>Leg press 1RM (kg)</td>
<td>16 (1, 31)⁺</td>
<td>19 (4, 36)⁺</td>
<td>35 (20, 50)⁺</td>
</tr>
<tr>
<td>Leg press 1RM (kg kg BM⁻¹)</td>
<td>0.2 (0.0, 0.4)⁺</td>
<td>0.2 (0.0, 0.4)⁺</td>
<td>0.4 (0.2, 0.6)⁺</td>
</tr>
<tr>
<td>Leg extension 1RM (kg)</td>
<td>8 (0, 16)⁺</td>
<td>3 (-5, 11)⁺</td>
<td>11 (3, 19)⁺</td>
</tr>
<tr>
<td>Leg extension 1RM (kg kg BM⁻¹)</td>
<td>0.1 (0.0, 0.2)⁺</td>
<td>0.0 (0.0, 0.0)⁺</td>
<td>0.1 (0.0, 0.2)⁺</td>
</tr>
<tr>
<td>Bench press 1RM (kg)</td>
<td>3 (-3, 8)⁺</td>
<td>-1 (-7, 4)⁺</td>
<td>1 (-4, 6)⁺</td>
</tr>
<tr>
<td>Bench press 1RM (kg kg BM⁻¹)</td>
<td>0.0 (0.0, 0.1)⁺</td>
<td>0.0 (-0.1, 0.0)⁺</td>
<td>0.0 (-0.1, 0.0)⁺</td>
</tr>
<tr>
<td>Muscle thickness (cm)</td>
<td>0.0 (-0.1, 0.1)⁺</td>
<td>0.0 (-0.1, 0.1)⁺</td>
<td>0.0 (-0.1, 0.1)⁺</td>
</tr>
<tr>
<td>VO₂peak (L·min⁻¹)</td>
<td>0.3 (0.2, 0.4)⁺</td>
<td>-0.1 (-0.2, 0.0)⁺</td>
<td>0.2 (0.0, 0.3)⁺</td>
</tr>
<tr>
<td>VO₂peak (mL·kg⁻¹·min⁻¹)</td>
<td>3.5 (2.0, 5.0)⁺</td>
<td>-2.0 (-3.0, -0.1)⁺</td>
<td>2.0 (0.1, 3.0)⁺</td>
</tr>
<tr>
<td>MAP (W)</td>
<td>26 (18, 34)⁺</td>
<td>-4 (-11, 4)⁺</td>
<td>22 (15, 30)⁺</td>
</tr>
<tr>
<td>MAP (W kg(^{-1}))</td>
<td>-0.1 (0.2, 0.4)</td>
<td>0.2 (0.1, 0.3)</td>
<td>0.2 (0.3, 0.5)</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>REE (kJ d(^{-1}))</td>
<td>-6 (-104, 91)</td>
<td>-42 (-147, 63)</td>
<td>-48 (-153, 57)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol L(^{-1}))</td>
<td>-0.2 (-0.5, 0.2)</td>
<td>0.3 (-0.5, 0.5)</td>
<td>0.1 (-0.2, 0.4)</td>
</tr>
<tr>
<td>AUC(_{total}) glucose (mmol h(^{-1})L(^{-1}))</td>
<td>-68 (-153, 16)</td>
<td>65 (-154, 24)</td>
<td>3 (-86, 92)</td>
</tr>
<tr>
<td>Fasting plasma insulin (mIU L(^{-1}))</td>
<td>-1.4 (-3.6, 0.8)</td>
<td>0.8 (-3.2, 0.5)</td>
<td>-0.6 (-2.9, 1.8)</td>
</tr>
<tr>
<td>AUC(_{total}) insulin (mmol h(^{-1})L(^{-1}))</td>
<td>-1 (-1879, 1881)</td>
<td>964 (-1085, 3013)</td>
<td>963 (-1086, 3012)</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>-0.2 (-0.5, 0.0)</td>
<td>0.1 (-0.1, 0.4)</td>
<td>-0.1 (-0.3, 0.2)</td>
</tr>
<tr>
<td>Steps (steps d(^{-1}))</td>
<td>2077 (-183, 4337)</td>
<td>-1811 (-4071, 449)</td>
<td>266 (-1994, 2526)</td>
</tr>
</tbody>
</table>

ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. 1RM, one-repetition maximum; BMI, body mass index; CSA, muscle fiber cross-sectional area; HOMA2-IR, homeostatic model assessment of insulin resistance; MAP; maximal aerobic power; REE, resting energy expenditure. \(^a\), \(P<0.05\) for Pre-Post change within group; \(^b\), \(P<0.05\) for Post-DT change within group; \(^c\), \(P<0.05\) for Pre-DT change within group.

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Table S2. Baseline physical activity (Pre) and changes following 6 weeks of exercise training (Post) and 2.5 weeks of detraining (DT) in middle-aged men.

<table>
<thead>
<tr>
<th></th>
<th>ENT</th>
<th></th>
<th>HIIT</th>
<th></th>
<th>RET</th>
<th></th>
<th>Main effects (P)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Week 4</td>
<td>DT</td>
<td>Pre</td>
<td>Week 4</td>
<td>DT</td>
<td>Pre</td>
<td>Week 4</td>
<td>DT</td>
<td>Time</td>
<td>Group</td>
</tr>
<tr>
<td>Steps (steps d⁻¹)</td>
<td>8907 ± 11192</td>
<td>9072 ± 9385</td>
<td>10409 ± 9672</td>
<td>10134 ± 9875</td>
<td>9560 ± 9072</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.067</td>
<td>1.000</td>
</tr>
<tr>
<td>Moving (% d⁻¹)</td>
<td>2163 ± 3495</td>
<td>2699 ± 1324</td>
<td>2847 ± 2322</td>
<td>3180 ± 1787</td>
<td>2296 ± 2322</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.011</td>
<td>0.862</td>
</tr>
<tr>
<td>Stepping (% d⁻¹)</td>
<td>14 ± 7</td>
<td>16 ± 7</td>
<td>13 ± 3</td>
<td>15 ± 2</td>
<td>14 ± 4</td>
<td>15 ± 3</td>
<td>13 ± 3</td>
<td>0.011</td>
<td></td>
<td>0.048</td>
<td>0.991</td>
</tr>
<tr>
<td>Cycling (% d⁻¹)</td>
<td>1 ± 1</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>1 ± 0</td>
<td>0 ± 1</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>&lt;0.001</td>
<td></td>
<td>0.023</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Standing (% d⁻¹)</td>
<td>26 ± 9</td>
<td>24 ± 10</td>
<td>22 ± 6</td>
<td>24 ± 9</td>
<td>26 ± 8</td>
<td>22 ± 7</td>
<td>26 ± 11</td>
<td>27 ± 11</td>
<td>0.023</td>
<td>0.011</td>
<td>0.772</td>
</tr>
<tr>
<td>Sitting (% d⁻¹)</td>
<td>60 ± 15</td>
<td>59 ± 13</td>
<td>65 ± 8</td>
<td>64 ± 11</td>
<td>59 ± 9</td>
<td>64 ± 11</td>
<td>60 ± 13</td>
<td>60 ± 12</td>
<td>0.011</td>
<td>0.613</td>
<td>0.261</td>
</tr>
<tr>
<td>Total time awake (h d⁻¹)</td>
<td>14 ± 4</td>
<td>14 ± 3</td>
<td>15 ± 3</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>13 ± 1</td>
<td>15 ± 2</td>
<td>14 ± 2</td>
<td>0.917</td>
<td>0.627</td>
<td>0.732</td>
</tr>
</tbody>
</table>

Values are mean ± SD. ENT, endurance exercise training; HIIT, high-intensity interval training; RET, resistance exercise training. d, day. a, P<0.05 vs Pre within group; b, P<0.05 vs Post within group; *, P<0.05 vs ENT at week 4. Moving = stepping + cycling.