Title: Skeletal muscle adaptive responses to different types of short-term exercise training and detraining in middle-aged men

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## Supplementary Material

## 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 ºC for 10 min at 4,095 rpm and plasma aliquots were stored at -80ºC 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 (W.kg-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.

### 1RM 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.

## 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.

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**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 VO2peak and relative maximal aerobic power

At baseline, there were no significant differences in relative VO2peak or relative maximal aerobic power (MAP). A significant *time × group* interaction effect (*P*=0.002) was observed for change in relative VO2peak (**Figure S2A**). In response to exercise training, relative VO2peak increased significantly for HIIT (+4.1 ± 2.7 mL.kg-1.min-1, *P*<0.001) and ENT group (+3.5 ± 2.9 mL.kg-1.min-1, *P*<0.001), but not for RET. After detraining, relative VO2peak decreased compared to post-exercise training for HIIT (-1.9 ± 1.7 mL.kg-1.min-1, *P* = 0.029) and ENT (-1.7 ± 1.7 mL.kg-1.min-1, *P*=0.024). After detraining, relative VO2peak remained elevated compared to baseline for HIIT (+2.3 ± 2.8 mL.kg-1.min-1, *P*=0.024) and ENT (+1.8 ± 1.9 mL.kg-1.min-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.

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