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Resistance training load does not determine resistance training-induced hypertrophy across upper and lower limbs in healthy young males

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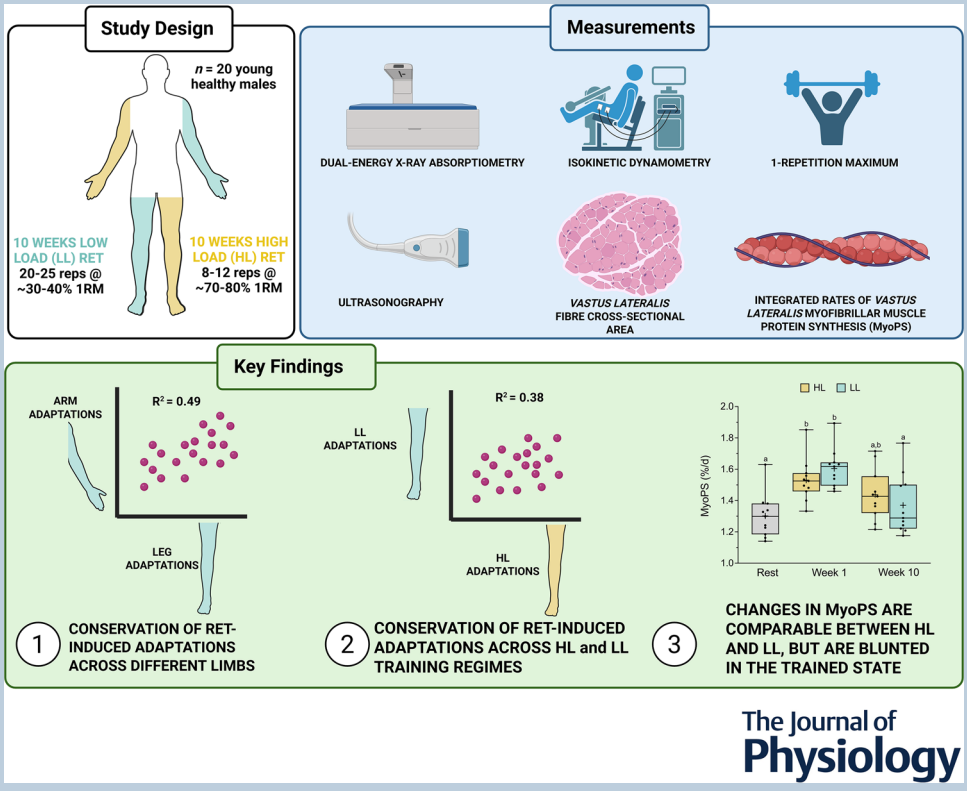

Abstract figure legend In healthy young males, we found that skeletal muscle hypertrophy following a period of resistance exercise training (RET) was consistent within and between subjects, as assessed using a variety of established methods. The skeletal muscle hypertrophic response was independent of external load. Changes in myofibrillar muscle protein synthesis (MyoPS) rates were comparable between higher and lower loads but were blunted following a period of RET despite progressive overload. Our findings show that when higher- and lower-load RET is performed to volitional fatigue, neither load nor limb location mediates the RET-induced hypertrophic response, but there is a relatively conserved RET-induced training response within an individual.

M. J. Lees and J. C. Mcleod have contributed equally to this work.

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Abstract Resistance exercise training (RET) leads to marked interindividual heterogeneity in the hypertrophic response. Whether such heterogeneity is due to endogenous (i.e. inherent biological factors) or exogenous variables (i.e. external load) has not been firmly established. Twenty healthy young male participants completed thrice-weekly resistance exercise sessions for 10 weeks. Each participant had their legs and arms randomly assigned to perform unilateral bicep curls or knee extensions with either a higher (heavier) load (HL: 8–12 repetitions; ∼70%–80% of one-repetition maximum (1RM)) or, in the contralateral limb, lower load (LL: 20–25 repetitions at ∼30%–40% 1RM) for three sets to volitional fatigue during each session. Fat- and bone-free mass (dual-energy X-ray absorptiometry), muscle size (ultrasonography and muscle biopsies) and strength were measured pretraining and at 10 weeks. Skeletal muscle biopsies were obtained from the vastus lateralis, and we used ingested deuterated water to assess myofibrillar protein synthesis (MyoPS) at weeks 1 and 10 during training. Despite considerable interindividual variability in hypertrophic responses, we observed that muscle hypertrophy following RET was relatively well conserved within *versus* between subjects and was unaffected by load. Rates of MyoPS in weeks 1 and 10 of training were increased relative to rest (Week 1: $\Delta 0.27 \pm 0.11$, $P < 0.0001$; Week 10: $\Delta 0.10 \pm 0.14$%/d, $P = 0.009$); however, MyoPS was attenuated in week 10 *versus* week 1 ($\Delta 0.16 \pm 0.18$%/d, $P < 0.001$). MyoPS rates were less heterogenous within *versus* between individuals. Variation in RET-induced muscle hypertrophy occurred independent of external load and was relatively well conserved (i.e., retention of the hypertrophic response) across different anatomical limbs within an individual.

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Key points

- Considerable interindividual variability exists in resistance exercise training (RET)-induced muscle hypertrophy. However we observed that RET-induced muscle hypertrophy is relatively conserved within an individual (i.e. between the upper- and lower body) and is independent of external load when RET is performed to volitional fatigue.
- Changes in myofibrillar protein synthesis (MyoPS) rates are comparable with both higher and lower loads but are blunted following a period of RET despite progressive overload.
- There is negligible shared variance between RET-induced increases in muscle size and strength. Additionally, there are limited relationships between measures used to assess RET-induced muscle hypertrophy.
- We conclude that when effort is matched (i.e. working to volitional muscular fatigue), RET-induced hypertrophy is mediated to a far greater degree by inherent endogenous biological factors, which account for a large proportion of the heterogeneity between individuals.

Matthew Lees is a postdoctoral research fellow in the Exercise Metabolism Research Group at McMaster University (Hamilton, Ontario, Canada). His passions lie in leveraging whole-body and cellular methods to answer important questions in muscle physiology, nutrition, ageing and physical (in)activity. Presently, his research investigates the impact of nutrition and exercise on the regulation of skeletal muscle mass in both youth and ageing. **Jonathan Mcleod** is a postdoctoral research fellow in the Nutrition and Metabolism Research Group at Queen's University (Kingston, Ontario, Canada). His research leverages stable isotope tracers and mass spectrometry to investigate human skeletal muscle protein metabolism under clinically relevant conditions such as disuse and bed rest. Jonathan's work aims to improve our understanding of the metabolic changes occurring during periods of inactivity and their implications for health and recovery.

Introduction

Skeletal muscle exhibits remarkable phenotypic plasticity in response to changing contractile stimuli (Joanisse et al., 2020). Resistance exercise training (RET) increases the size and force-generating capacity of skeletal muscle by imposing mechanical tension on the contractile apparatus. The accretion of skeletal muscle tissue that occurs following RET has been classically referred to as skeletal muscle hypertrophy, a process defined as the axial increase in the cross-sectional area (CSA) of a muscle or muscle fibre in response to loading (Lim et al., 2022). Various methods may be used to assess this at the whole-body and fibre-specific levels, such as magnetic resonance imaging (MRI), computed tomography, ultrasound and biopsy sampling to determine muscle fibre CSA (Haun et al., 2019). Recent deep phosphoproteomic analyses revealed that RET promotes the activation of a specific signalling pathway involving MKK3b/6, p38, MK2 and mTORC1 in skeletal muscle (Zhu et al., 2025). The RET-induced activation of this pathway is robustly associated with myofibrillar protein synthesis (MyoPS), leading to myofibrillogenesis. This ultrastructural adaptation to loading represents the primary mediator driving the axial expansion of skeletal muscle (Jorgenson et al., 2024; Zhu et al., 2025).

The hypertrophic response of skeletal muscle to contractile loading in humans is characterised by substantial interindividual variability (Ahtiainen et al., 2016; Charbonneau et al., 2008; Davidsen et al., 2011; Erskine, Williams et al., 2014; Hubal et al., 2005; Lavin et al., 2021; Morton, Sato et al., 2018; Roberts, Haun et al., 2018; Roberts, Romero et al., 2018). To some degree this interindividual variability is mediated to a small extent by exogenous factors, such as protein intake (Morton, Murphy et al., 2018) and manipulating training-associated variables such as volume (Cunha et al., 2020; Currier et al., 2023; Mcleod et al., 2024; van Vossel et al., 2023), frequency (Currier et al., 2023; Schoenfeld et al., 2019) and/or exercise selection (Currier et al., 2023; McLeod et al., 2024; Paoli et al., 2017). Recent work has shown that interindividual differences in hypertrophy could be underpinned by intrinsic features of skeletal muscle, such as baseline protein-coding gene expression (Lavin et al., 2021), myonuclei, capillaries and immune cells (Long et al., 2022), but are not mediated by muscle fibre typology (van Vossel et al., 2023).

Skeletal muscle hypertrophy is a hallmark of RET. Efforts have been made to reveal the factors underpinning this response, as well as the determinants of the heterogenous hypertrophic response across individuals (Davidsen et al., 2011; Petrella et al., 2008; Stec et al., 2017; Stokes et al., 2020; van Vossel et al., 2023). Although it is unlikely that an individual could be a 'non-responder' to RET at least from a strength standpoint (Churchward-Venne et al., 2015), it is also evident that individuals show considerable interindividual variability in RET-induced adaptations, including hypertrophy and strength gains (Charbonneau et al., 2008; Davidsen et al., 2011; Erskine, Williams et al., 2014; Roberts, Haun et al., 2018; Roberts, Romero et al., 2018). The reproducibility of the hypertrophic response, as a hallmark adaptation to RET, is also questionable (Mattioni Maturana et al., 2021; Ross et al., 2019); in other words are responders always responders? We and others have proposed that inherent endogenous factors (i.e. a person's inherent biological response) represent the greatest source of interindividual variability in RET-induced skeletal muscle hypertrophy (Joanisse et al., 2020; Lim et al., 2022; Roberts et al., 2023), as opposed to the manipulation of exogenous RET variables.

In the present study, we aimed to determine the relative effect of external load (i.e. an exogenous factor) and limb location (upper- *versus* lower body; an endogenous factor) on RET-induced changes in skeletal muscle size and strength. We used a unilateral, within-subject design to assess the impact of external load (higher load (HL) *versus* lower-load (LL)) and limb location (upper- *versus* lower body) on skeletal muscle hypertrophy and strength following 10 weeks of RET performed to volitional fatigue. A within-subject design, utilising anatomically distinct muscle groups, enables the assessment of skeletal muscle plasticity between muscle groups within the same individual, thereby increasing the likelihood of observing any conservation of the RET response (MacInnis et al., 2017; Stokes et al., 2020). We hypothesised that the within-subject response (i.e. the intraindividual variability of skeletal muscle hypertrophy between different limbs using different loads) would be significantly lower than the characteristic heterogeneous responses between participants. Such a scenario would indicate that endogenous mechanisms are likely more important drivers of load-induced muscle hypertrophy.

Methods

Participants

Healthy, recreationally active but untrained young males (n = 20; see Table 1 for baseline descriptive characteristics) participated in this study. The study was approved by the Hamilton Integrated Research Ethics Board (#4774) and registered at ClinicalTrials.gov as NCT03993483. The study conformed to the standards for the use of human subjects in research as outlined by the Canadian Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2, 2022 (https://ethics.gc.ca/eng/policy-politique_tcps2-eptc2_2022.html) and the Declaration of Helsinki

Table 1. Baseline participant characteristics

Variable	Mean ± SD
Age (years)	22 ± 3
Height (cm)	181 ± 7
Weight (kg)	85 ± 24
BMI (kg/m^2)	26.2 ± 6.1
Whole-body FBFM (kg)	60 ± 11
Appendicular FBFM (kg)	29 ± 6
Biceps curl 1RM (kg)	18 ± 4
Knee extension 1RM (kg)	40 ± 14
VL CSA (cm^2)	23 ± 7
BB CSA (cm^2)	11 ± 3
VL thickness (mm)	2.7 ± 0.6
BB thickness (mm)	2.5 ± 0.5
Type I fibre CSA (μm^2)	5984 ± 1184
Type II fibre CSA (μm^2)	6669 ± 1831
Type IIa fibre CSA (μm^2)	6907 ± 1885
Type IIx fibre CSA (μm^2)^A	6594 ± 1695
Type IIa/x fibre CSA (μm^2)^B	6509 ± 1754
Type I fibre distribution (%)	35 ± 1
Type IIa fibre distribution (%)	45 ± 1
Type IIx fibre distribution (%)	4 ± 5
Type IIa/x fibre distribution (%)	15 ± 5

Note: Values are expressed as mean ± SD (n = 20). $^A N$ = 18; $^B N$ = 12.
Abbreviations: 1RM, one-repetition maximum; BB, biceps brachii; BMI, body mass index; CSA, cross-sectional area; FBFM, fat and bone-free (lean) mass; VL, vastus lateralis.

(https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). Each participant was informed of the purpose of the study, experimental procedures and potential risks before written informed consent was obtained.

Study overview

Each participant's arm and leg (based on one-repetition maximum [1RM] strength) were randomly assigned to either a higher-load, lower-repetition RET (HL: 8–12 repetitions at ∼70%–80% 1RM) or lower-load, higher-repetition (LL: 20–25 repetitions at ∼30%–40% 1RM) group in a counterbalanced manner. The contralateral limbs were assigned to the opposite condition, such that the training load was randomised and counterbalanced irrespective of limb dominance.

Each participant completed three supervised RET sessions each week (Monday, Wednesday and Friday) for 10 weeks (Fig. 1). Each RET session consisted of three sets of unilateral knee extensions and dumbbell preacher curls, allocated to each limb according to its condition (HL or LL). For each RET session, knee extensions were performed before dumbbell preacher curls, but the starting limb for each exercise was randomised across RET sessions. Each set was separated by 90 s of rest, and each repetition was performed to a cadence of 2:0:2 s (eccentric: isometric pause: concentric) with proper form (i.e. full range of motion, remaining seated and keeping the chest and elbow in contact with the apparatus' pads during dumbbell preacher curls). For each set, participants were instructed to achieve volitional fatigue, defined as the inability to complete another concentric muscle action through the full range of motion with proper form. Compliance for the training sessions was 100%. Loads for each set were adjusted to ensure that volitional fatigue was reached within 8–12 and 20–25 repetitions for the HL and LL limbs, respectively. If participants completed more repetitions than the upper limit of the given repetition range during the first set of a training session, the load was increased for the first set of the subsequent training sessions.

Dietary consideration

Using dietary logs, participants were asked to provide a dietary recall of all food and drink consumed over 3 days during familiarisation (Week 0), Week 1 and Week 10. Dietary recall was analysed using NutriBase dietary analysis software (NutriBase 11 Professional Edition, version 11.5, Cybersoft Inc., Phoenix, Arizona, USA). In addition, participants received 25 g of whey protein isolate (Leprino Foods, Denver, CO, USA) twice daily (post-exercise and pre-sleep) for the duration of the study to ensure that each participant was receiving adequate (>1.6 g/kg of body mass/day) daily protein intake (Morton, Murphy et al., 2018). There was no change in protein (25 ± 11% of total energy intake), carbohydrate (44 ± 11% of total energy intake) or fat (34 ± 10% of total energy intake) consumption throughout the study (detailed data not presented).

Muscle strength

Muscle strength was measured before the first RET session and 72 h following the last RET session using maximum voluntary contractions (MVC) and 1RMs. The MVC consisted of three 5 s isometric, unilateral attempts on each limb (knee extension (60°) and elbow flexion (110°); System 3, Shirley, NY, USA) with 120 s rest in between each attempt. Participants were familiarised with executing an MVC before obtaining baseline measurements. Further unilateral knee extension (Atlantis Inc., Laval, QC, Canada) and unilateral dumbbell preacher curl (York Barbell, York, PA, USA) 1RM were recorded before the first RET session after a general warm-up (5 min on a cycle ergometer) with load progressively increasing (∼4.5 kg [knee extension] or

∼2.5 kg [dumbbell preacher curl]) and the number of repetitions decreasing (∼8, 5, 3 and then 1) with 120 s of rest between each set until the participant could not perform a single successful repetition. For knee extensions, successful repetitions were defined as starting at 90° and finishing at 170° with their hips in contact with the seat. For dumbbell preacher curls, successful repetitions were defined as starting at 180° and finishing at complete elbow flexion, with the hips remaining in the seat, the chest against the apparatus and the elbow in contact with the pad.

Fat- and bone-free (lean) mass

Fat- and bone-free (lean) mass (FBFM) was assessed using dual-energy X-ray absorptiometry (DXA) on a Lunar iDXA total body scanner (GE Medical Systems, Madison, WI, USA) following an overnight fast (pre- and post-intervention) and 72 h following their last exercise bout (postintervention). Data were analysed in the medium scan mode (Lunar enCORE version 14.1, GE Medical Systems). Each analysis region (i.e. head, torso, arms and legs) was partitioned by the software before a manual inspection by a blinded study investigator. The coefficient of variation (CV) for FBFM based on repeat scans in our laboratory was less than 1.6%.

Muscle thickness and cross-sectional area

Muscle thickness and CSA were assessed using B-mode ultrasonography (US) and were performed by the same technician pre- and posttraining, consistent with previous work from our laboratory (Stokes et al., 2021). Each participant lay supine for 10 min before a BK3500 unit and an 18L5 probe (BK Medical North America, Peabody, MA, USA) were used to measure muscle thickness and CSA. The settings were determined during pilot testing and remained constant at every measurement and within the same participant. In our laboratory, the intra-rater reliability for US analysis of muscle size over 10 weeks is good to excellent, with an intraclass correlation coefficient (ICC) of 0.940 (95% confidence interval (CI), 0.875–0.972). Interrater reliability is moderate to excellent (ICC = 0.847; 95% CI: 0.666–0.926). At all-times over 10 weeks, US was significantly and highly correlated (all $r > 0.85$) with MRI, the accepted 'gold standard' technique for measuring muscle size (Stokes et al., 2021).

Each video file was converted to TIFF frames using Filezigzag (www.filezigzag.com) before being stitched into a panoramic image with Autostitch (http://matthewalunbrown.com/autostitch/autostitch.html). To evaluate the validity of Autostitch 20 unstitched sets of tiff frames (5 baseline biceps brachii (BB), 5 posttraining BB, 5 baseline vastus lateralis (VL), 5 post-training VL) were manually stitched into a panoramic image using GIMP (www.gimp.org), similar to manual stitching methodologies reported elsewhere for quality control (Jakubowski et al., 2019; Lixandrao et al., 2014). The panoramic images were uploaded and calibrated using ImageJ (National Institutes of Health, Bethesda, MD, USA) before being assessed for CSA (using the polygon tracing tool) and muscle thickness (MT; a

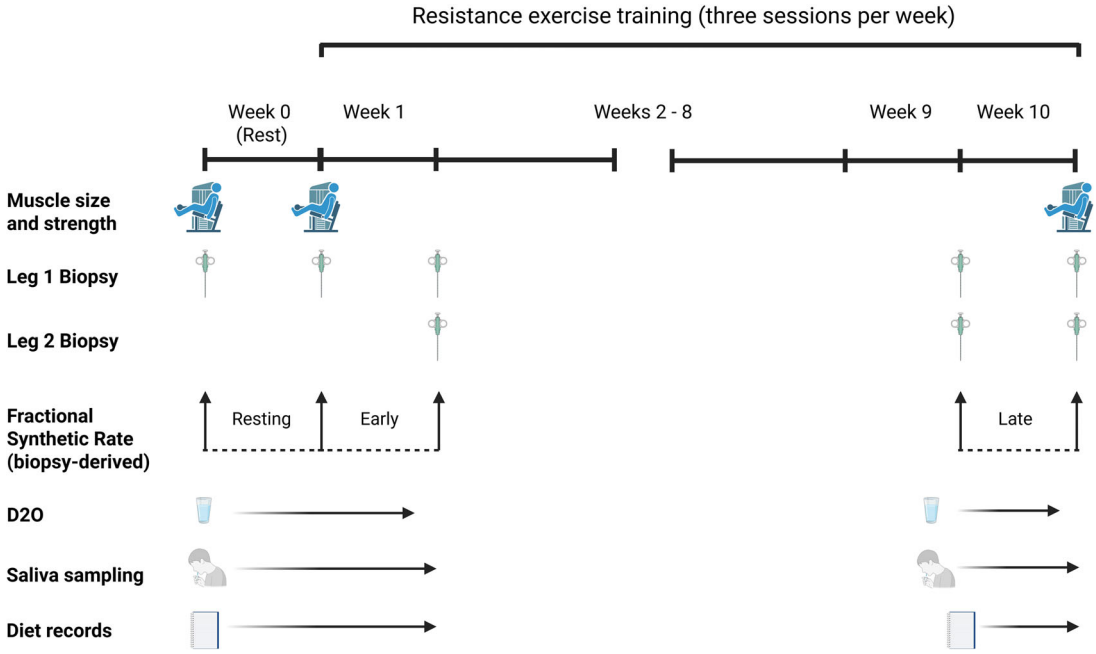

Figure 1. Schematic overview of the study protocol
Created in BioRender. Lees, M. (2025) https://BioRender.com/jpemzbs.

straight line drawn at the thickest part [muscle belly] of each image). All images were stitched and analysed by an experienced examiner in a blinded manner. Each measurement, for each limb, was performed in duplicate (separately captured and stitched) and showed high ICCs (specifications: two-way mixed, single-measures, consistency) for both the VL (CSA: 0.89; MT: 0.73) and BB (CSA: 0.92; MT: 0.83). The previously published SEM and minimal detectable change for our image stitching technique are 1.09 cm^2 and 3.03 cm^2, respectively (Stokes et al., 2021).

Muscle fibre cross-sectional area

Skeletal muscle biopsies were obtained from the VL using a customised Bergström needle modified for manual suction under local anaesthesia (1% xylocaine). The unilateral skeletal muscle biopsy obtained at baseline and the bilateral biopsies obtained at the end of week 10 were used for CSA analysis, respectively. Approximately 30 mg from each biopsy was cleared of connective tissue, mounted in optimal cutting temperature medium and then frozen in liquid nitrogen-cooled isopentane. The samples were stored at −80°C until further analysis. Serial cross-sections were prepared (∼5 μm) using a Microm HM550 Cryostat (Thermo Fisher Scientific, Waltham, MA), with care taken not to expose samples to freeze-thaw cycles. Antibodies for dystrophin (MANDYS1 [3B7]), MHC I (BA-F8), MHC IIA/X (BF-35) and MHC IIX (6H1; all obtained from Developmental Studies Hybridoma Bank, Iowa, USA) were combined with secondary isotype-specific antibodies (Alexa Fluor 350 [#A21120], 488 [#A21131], 594 [#A21125] and 647 [#A21238]; Invitrogen, Thermo Fisher Scientific) before being mounted with Prolong Diamond Antifade Reagent (Life Technologies, Toronto, ON, Canada), similar to previous publications from our group (Jakubowski et al., 2019; Morton et al., 2016). Slides were kept in a dark drawer before being imaged the following day at wavelengths 350 nm (type IIA myofibres), 488 nm (dystrophin), 594 nm (type IIX myofibres) and 647 nm (type I myofibres) at 200× magnification with a Nikon Eclipse 90i fluorescent microscope (Nikon Instruments, Melville, NY, USA). Each dystrophin border was circled using the NIS-Elements software (Nikon Instruments) and exported as an individual region of interest (ROI) to enable the determination of fibre type and CSA. The cut-off for inclusion in our CSA analysis was a circularity of >0.80, which rendered 117 ± 63 fibres per biopsy. In addition, we quantified the type of each fibre based on relative stain intensity (i.e. relative expression of each MHC isoform), which provided an objective way to determine hybrid fibre types. Given the inherently low prevalence of type IIx and IIa/x fibres in healthy young males – and their further reduction with resistance training – some biopsies contained few or no fibres of these subtypes. We therefore report IIx and IIa/x CSA values for completeness and transparency, consistent with standard practice in muscle histology, but we recognise that their low fibre counts limit interpretability. Importantly, subtype-specific CSA values were not used in any inferential analyses or outcome rankings; only pooled type II CSA contributed to the hypertrophy composite. Thus, although these data provide biologically relevant context regarding fibre-type transitions, all primary conclusions rely on fibre groups with sufficient representation across participants. All image analyses were performed by a single-blinded study investigator.

Outcome ranking

Given the varying nature of the methods used to quantify hypertrophy at 'macroscopic' and 'microscopic' levels, and in a similar manner to prior work (Long et al., 2022), we defined an aggregate outcome ranking as follows. To assess the relative response of each participant (and in each limb) we ranked individuals based on the magnitude of change in outcomes that represent changes in 'muscle' that are commonly employed in studies of RET (appendicular FBFM [via DXA; arms and legs; HL and LL], muscle CSA [via US; arms and legs; HL and LL], muscle thickness [via US; arms and legs HL and LL], type I fibre CSA [via biopsy; legs only; HL and LL] and type II fibre CSA [via biopsy; legs only; HL and LL]). Although we present data on type I and pooled type II myofibre and subtype distributions, only type I CSA and pooled type II CSA were used in the outcome ranking calculation. For each outcome we assigned a rank of 1 to the individual with the greatest (most positive) change, and the smallest change was assigned a rank of 20. To broadly compare the upper-body *versus* lower-body responses for everyone, we summed the rank of each outcome in each condition (HL and LL) in the upper- and lower-body, respectively. Similarly to broadly compare an individual's hypertrophic response *versus* strength, we summed the rank of each outcome in each limb for hypertrophy and strength, respectively. We also compared individual outcomes between limbs, load, hypertrophy and strength.

D_2O labelling and measurement of protein synthesis

Deuterated water (^2H_2O) was used to label newly synthesised myofibrillar proteins (Wilkinson et al., 2014). After the first biopsy at Week 0, participants consumed eight aliquots of 70% ^2H_2O (1 ml/kg of FBFM; Cambridge Isotope Laboratories, Andover, MA, USA) every 90 min until 8 boluses were consumed (loading phase). After the loading phases at Week 0 and Week 9 participants

consumed one daily maintenance dose (1 ml/kg of FBFM) each morning. Saliva samples were collected using salivettes (Sarstedt AG & Co, Germany) before each loading phase and daily thereafter. Specifically participants were instructed to collect saliva samples each morning before brushing their teeth and taking their daily maintenance dose. Saturated salivettes were spun at 1000 *g* for 5 min to collect saliva at the bottom of each salivette. Each saliva sample was diluted 1:35 with distilled water in autosampler vials. ^{2}H (D) enrichment in the saliva samples was detected using cavity ring-down spectroscopy (Picarro L2130-i analyser, Picarro, Santa Clara, CA). Six injections were performed per sample, and the average of the last three δ2H measurements was used for data analysis (CV $\leq$0.5%). The measurements were then converted to atom percent excess (APE), as previously described for protein synthesis calculations (Wilkinson et al., 2014).

Integrated myofibrillar protein synthesis

Approximately 50 mg of muscle was homogenised in buffer (10 µl/mg 25 mM Tris, 0.5% v/v Triton X-100, with protease/phosphatase inhibitor tablets [Complete Protease inhibitor Mini-Tabs; Roche, and PhosStop; Roche Applied Science]) before centrifugation at 1500 *g* for 10 min at 4°C. The pellet was solubilised and centrifuged as previously described (Dufner et al., 2005). Myofibrillar proteins were precipitated in 1 mL of 1 M perchloric acid and washed twice with 70% ethanol. The remaining myofibrillar pellet was hydrolysed in 0.5 M HCl at 110°C for 72 h, and then protein-bound amino acids were purified by ion exchange chromatography in Dowex H^{+} resin (50WX8-200 resin, Sigma-Aldrich). Myofibrillar ^{2}H-alanine enrichments were determined as previously described (MacDonald et al., 2013). The integrated myofibrillar protein synthetic rate (%/d) was calculated as the incorporation of deuterium-labelled alanine into protein via deuterated water in accordance with Wilkinson et al. (2014):

$$\text{Fractional synthetic rate (FSR; \%/d)} = \left(\Delta \text{APE}_{\text{Ala}}/\text{APEp} \times \text{t}\right) \times 3.7 \times 100$$

where $\Delta \text{APE}_{\text{Ala}}$ is the change in deuterium-labelled, protein-bound alanine between time points, APE_{p} is the mean precursor (saliva enrichment) over time (t), multiplication by 3.7 adjusts for the mean number of ^{2}H atoms that can be incorporated into alanine, and multiplication by 100 converts the values to percentages (McGlory et al., 2018; McKendry et al., 2024). For MyoPS, data are presented for 11 participants only due to limited tissue availability.

Statistical analyses

A one-way ANOVA, with time as the independent variable, was used to assess changes and differences in dietary outcomes, FBFM, appendicular FBFM and exercise volume load. Two-way repeated-measures ANOVA with time and condition as the within-subject variables was used for changes in 1RM, peak torque, whole-muscle CSA, MT, muscle fibre CSA and MyoPS rates. In the presence of significant main and/or interaction effects Tukey's honestly significant difference (HSD) *post hoc* tests were used to assess the differences between means. For outcomes evaluated in both limbs (volume load, FBFM, US CSA, US MT, 1RM, peak torque and MyoPS rates), between-limb differences were assessed using paired *t* tests. All ANOVA, *t* tests and correlations were performed using SPSS (version 20; IBM Corp., Armonk, NY, USA). Student's *t* tests were carried out on the CV for each outcome (e.g. FBFM, US MT, US CSA and fibre CSA) and used to determine if there was a significant difference between the CV in the arms *versus* legs or on average (Table 2). To calculate the within-subject %CV for each hypertrophy assessment method, we first divided the SD by the mean across two measurement time points for each subject. These values were then converted to percentages and averaged to obtain the within-subject %CV. The between-subject %CV was obtained by calculating the SD of these means for each participant, dividing them by the 'mean of means' and expressing this as a percentage.

Linear regressions were used to detect shared variance between each participant's upper- and lower-body measurements, as well as between each participant's changes in muscle size and strength. Pearson's correlations and CV were used to assess variability across and within outcomes and participants. All regressions were performed using GraphPad Prism (version 9; La Jolla, CA, USA). Statistical significance was set at $P < 0.05$. Where data are presented as box and whisker plots, the box is the interquartile range; the cross is the mean; the line is the median; the tails are the minimum and maximum values; and the shaded area is the cumulative measurement error. When data are presented as waterfall plots, the relative change for each participant is indicated by the same symbol across outcomes, and the shaded area represents cumulative measurement error. Tabular data are presented in the text as mean $\pm$ SD unless otherwise stated.

Results

Muscle hypertrophy and strength

For the legs, there were no significant differences ($P = 0.197$) in average volume load over 10 weeks of

Table 2. Coefficients of variation (%CV) for hypertrophy outcomes

Hypertrophy		Between-participant CV (%)	Within-participant CV (%)
FBFM	Arms	20.9	3.7
	Legs	19.4	2.5
	Mean	20.1	3.1
US CSA	Arms	27.3	9.1
	Legs	28.3	6.2
	Mean	27.8	7.7
US MT	Arms	17.2	5.8
	Legs	18.7	6.1
	Mean	17.9	5.9
Fibre CSA	Type I	15.9	13.7
	Type II	20.6	15.7
	Mean	18.2	14.7
		Mean overall CV (%)	
	Arms	19.2 ± 1.9	6.2 ± 2.8[a]
	Legs	20.6 ± 4.7	8.8 ± 5.6[b]
	Average	20.1 ± 3.7	7.9 ± 4.7[c]

Note: Between-participant *versus* within-participant using unpaired *t* test.
Abbreviations: CSA, cross-sectional area; FBFM, fat and bone-free (lean) mass; MT, muscle thickness; US, ultrasound.
[a] $P = 0.003$.
[b] $P = 0.007$.
[c] $P < 0.0001$.

training between LL (81,968 ± 26,924 kg) and HL (78,583 ± 22,984 kg) conditions. However, for the arms there were significant differences ($P < 0.001$) in average volume load over 10 weeks of training between the HR (58,756 ± 12,351 kg) and LR (38,334 ± 8496 kg) conditions. Ten weeks of RET resulted in increased DXA-derived FBFM (Fig. 2*A*), whole-muscle CSA (Fig. 2*B*), whole MT (Fig. 2*C*, and muscle fibre CSA (Fig. 2*D*). When data were pooled by limb location, irrespective of load there was an increase in leg FBFM (Δ0.30 ± 0.30 kg, $P < 0.0001$), arm FBFM (Δ0.17 ± 0.14 kg, $P < 0.0001$), BB CSA (Δ1.30 ± 0.94 cm^2, $P < 0.0001$), VL MT (Δ0.14 ± 0.25 cm, $P = 0.001$), BB MT (Δ0.10 ± 0.34 cm, $P < 0.0001$), type I fibre CSA (Δ549 ± 1381 μm^2, $P = 0.016$) and type II fibre CSA (Δ762 ± 1729 μm^2, $P = 0.008$) but not VL CSA (Δ0.72 ± 2.53 cm^2, $P = 0.078$). There were no significant differences between HL and LL for any outcome measure assessing muscle size (all $P > 0.05$; Fig. 2*A*–*D*; Table 3). The myofibre percentage distribution for type IIx and type IIa/x fibres significantly changed in both the HL (%type IIx: Δ−20.59 ± 37.34, $P = 0.003$; %type IIa/x: Δ40.46 ± 106.40, $P = 0.010$) and LL (%type IIx: Δ−23.62 ± 40.82, $P = 0.001$; %type IIa/x: Δ97.06 ± 127.88, $P = 0.014$) groups, respectively. By contrast the distribution of type I and type IIa myofibres did not change from baseline (Table 1) in the HL (%type I: Δ−0.00 ± 0.37, $P = 0.969$; %type IIa: Δ0.37 ± 2.61, $P = 0.570$) or LL (%type I: Δ0.15 ± 0.50, $P = 0.202$; %type IIa: Δ1.21 ± 4.84, $P = 0.288$) groups, respectively.

There were also limited relationships between the measurement techniques used to determine hypertrophy (delta change in each measure) at the arms (all $r < 0.192$, $P > 0.05$), as shown by Pearson's product-moment correlations. For the legs, US CSA was negatively correlated with type I ($r = -0.321$, $P = 0.044$) and type II fibre CSA ($r = -0.414$, $P = 0.008$), as was US MT ($r = -0.343$, $P = 0.032$; $r = -0.480$, $P = 0.002$). Both US measures at the legs were significantly associated ($r = 0.556$, $P < 0.0001$), as were type I and type II fibre CSA ($r = 0.790$, $P < 0.0001$); however, no other statistically significant associations were observed between measures.

There was an increase in biceps curl 1RM (HL: 3.8 ± 4.3, $P = 0.011$; LL: 3.2 ± 4.8 kg, $P = 0.004$), knee extension 1RM (HL: 24 ± 11, $P < 0.0001$; LL: 22 ± 9 kg, $P < 0.0001$), isometric elbow flexion peak torque (HL: 11 ± 10, $P = 0.003$; LL: 12 ± 15 Nm, $P < 0.001$) and isometric knee extension peak torque (HL: 38 ± 53, $P = 0.008$; LL: 42 ± 41 Nm, $P = 0.006$), with no differences between HL and LL (all $P > 0.05$; Table 3). In contrast to hypertrophy measures, there were strong, statistically significant relationships between measures of arm strength ($r = 0.728$, $P < 0.0001$) and leg strength ($r = 0.705$, $P < 0.0001$).

There was significant shared variance in RET-induced hypertrophy between the upper- and lower-body ($R^2 = 0.492$, $P < 0.001$) and between HL and LL conditions ($R^2 = 0.382$, $P = 0.004$; Fig. 2*E* and *F*) despite considerable heterogeneity in the hypertrophic

response. The relative (percentage) change was [mean (range)] 4.8% (−2.7% to 13%) for arm FBFM, 3.1% (−2.0% to 7.5%) for leg FBFM, 13.8% (1.1%–27.0%) for arm CSA, 4.7% (−10.2% to 18.9%) for leg CSA, 7.6% (−4.0% to 18.7%) for arm MT, 6.3% (−4.2 to 23.6%) for leg MT, 12.0% (−23.7% to 48.7%) for type I fibre CSA and 17.9% (−29.6% to 95.6%) for type II fibre CSA. Notably there was no association between baseline strength and the degree of the hypertrophic response (Fig. 3). The between-participant CV for each outcome was significantly greater than the within-participant CV for each outcome (see Table 2).

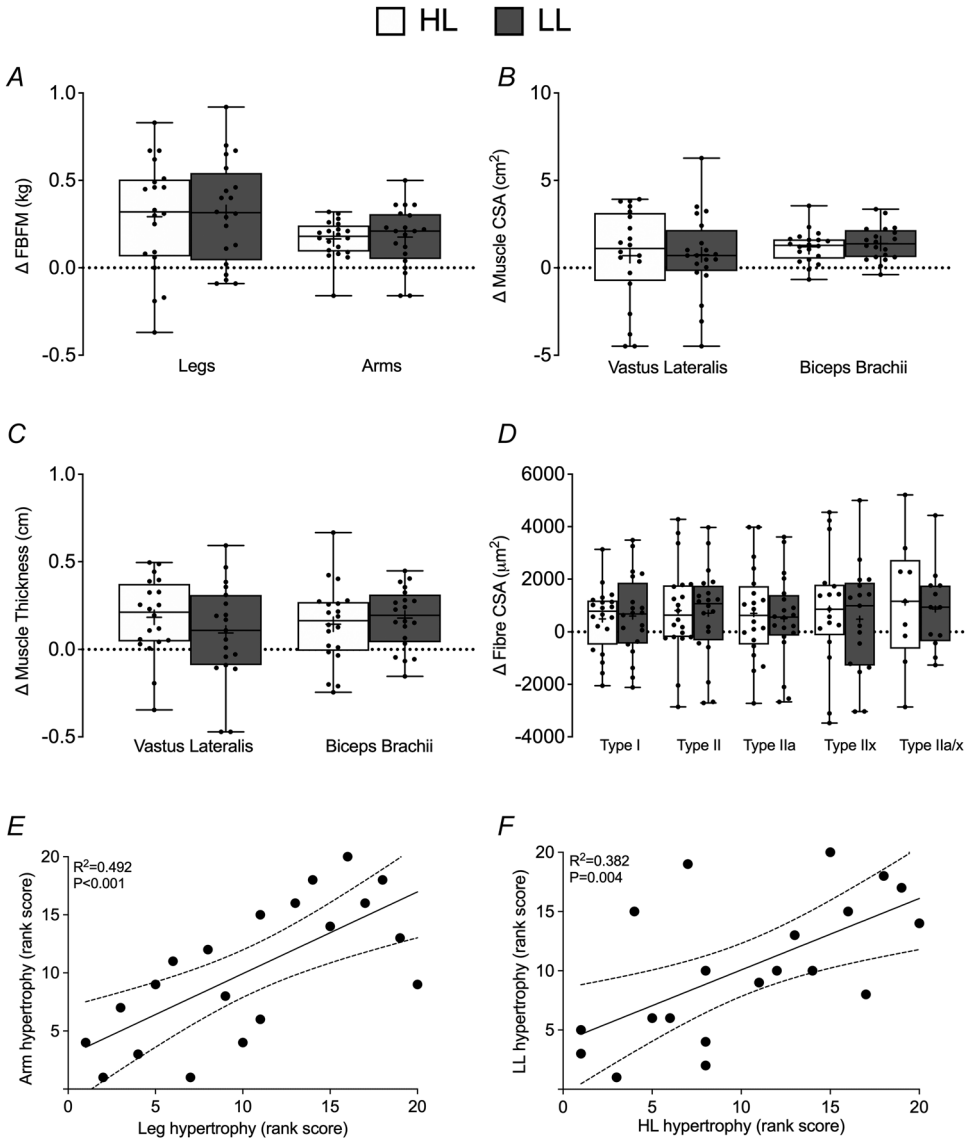

Figure 2. Ten weeks of resistance exercise training increased muscle size, independent of load or limb location (upper *versus* lower body), and the response was relatively conserved within an individual
A, fat- and bone-free mass (FBFM) measured using dual-energy X-ray absorptiometry in legs and arms; *B*, whole-muscle cross-sectional area (CSA) measured using ultrasonography in the vastus lateralis (VL) and biceps brachii (BB); *C*, whole-muscle thickness (MT) measured using ultrasonography in VL and BB; D, muscle fibre CSA from muscle biopsies of the VL measured using immunohistochemistry to identify type I and type II muscle fibres; *E*, ordinary least squares regression between changes in the upper- and lower body within each individual; *F*, ordinary least squares regression between the changes in lower load and higher load limbs within each individual. In panels *A–D* the box and whisker plots are presented, with the interquartile range (box), minimum and maximum values (error bars), mean (cross) and median (horizontal line), along with individual changes (dots). In panels *E* and *F* each individual's overall rank score for limb hypertrophy (*E*) and load (*F*) is represented by a dot, and a line of best fit with a 95% confidence interval is included as a solid and dotted line, respectively. In panels *A–D* within each limb paired *t* tests were used to assess differences between high load and low load.

Table 3. Muscle size and strength changes from baseline following high-lad (HL) and low-load (LL) resistance exercise training

Variable	High load (n = 20)	Low load (n = 20)	P-value
Hypertrophy			
Arm FBFM (kg)	0.17 ± 0.11	0.18 ± 0.18	0.778
Leg FBFM (kg)	0.29 ± 0.32	0.32 ± 0.29	0.766
BB thickness (cm)	0.14 ± 0.23	0.18 ± 0.17	0.556
VL thickness (cm)	0.18 ± 0.23	0.09 ± 0.28	0.312
BB CSA (cm^2)	1.20 ± 0.92	1.39 ± 0.98	0.518
VL CSA (cm^2)	0.69 ± 2.73	0.75 ± 2.43	0.932
Type I fibre CSA (μm^2)	495 ± 1244	602 ± 1537	0.726
Type IIa fibre CSA (μm^2)	704 ± 1754	510 ± 1672	0.967
Type IIx fibre CSA (μm^2)[A]	868 ± 2203	483 ± 2099	0.511
Type IIa/x fibre CSA (μm^2)[B]	1138 ± 2422	875 ± 1588	0.876
Type II fibre CSA (μm^2)	811 ± 1746	712 ± 1756	0.815
Strength			
Biceps curl 1RM (kg)	3.8 ± 4.3	3.2 ± 4.8	0.230
Knee extension 1RM (kg)	24 ± 11	22 ± 9	0.125
Elbow flexion MVC (Nm)	11 ± 10	12 ± 15	0.680
Knee extension MVC (Nm)	38 ± 53	42 ± 51	0.590

Note: Values are expressed as mean ± SD (N = 20). [A]N = 16; [B]N = 9. Paired t tests were used to assess differences between high load and low load.
Abbreviations: 1RM, one-repetition maximum; BB, biceps brachii; CSA, cross-sectional area; FBFM, fat and bone-free (lean) mass; MVC, maximal voluntary contraction; US, ultrasound; VL, vastus lateralis.

The percentage change in muscle strength following 10 weeks of RET ranged from −19% to 48% for elbow flexion peak torque, −23% to 70% for knee extension peak torque, −14% to 100% for dumbbell biceps curl 1RM and 10% to 177% for knee extension 1RM. The mean within-participant CV for strength across limbs and condition was 18.1 ± 9.9%, and the mean between-participant CV was 31.3 ± 13.8% ($P = 0.176$).

Interestingly there was no relationship between ranked scores of hypertrophy and strength (Fig. 4*A*); however there was a significant shared variance (correlation) between changes in upper- and lower-body strength ($R^2 = 0.351$, $P = 0.006$; Fig. 4*B*), as well as between strength rankings in the HL and LL conditions ($R^2 = 0.656$, $P < 0.0001$; Fig. 4*C*).

Integrated myofibrillar protein synthesis rates

Pooled MyoPS rates increased in the early and late phases of RET relative to rest (Week 1: Δ0.27 ± 0.11, $P < 0.0001$; Week 10: Δ0.10 ± 0.14 %/d, $P = 0.009$); however the increase was attenuated in the final week (Week 10 *versus* Week 1: Δ − 0.16 ± 0.18 %/d, $P < 0.001$). By condition elevated MyoPS rates were noted in HL ($P < 0.0001$) and LL ($P < 0.001$) in the early phase compared to rest, but by the late phase rates did not differ significantly from rest ($P = 0.108$, $P > 0.999$, respectively; Fig. 5*A*). In the LL condition MyoPS rates were significantly lower in the late phase than in the early phase ($P = 0.003$). There were no significant differences in MyoPS rates by load in either the early ($P = 0.602$) or late ($P > 0.999$) phases.

There was no significant shared variance (correlation) between MyoPS early (Week 1) *versus* late (Week 10) during RET ($R^2 = 0.133$, $P = 0.096$; Fig. 5*B*) or average (Week 1 and Week 10) MyoPS rates between HL and LL ($R^2 = 0.163$, $P = 0.063$; Fig. 5*C*).

Discussion

In the present study, we leveraged a range of whole-body and fibre-specific techniques to measure skeletal muscle hypertrophy in response to 10 weeks of HL and LL limb-specific RET and examine the relative conservation of the hypertrophic response within an individual. Consistent with previous research (Currier et al., 2023; Schoenfeld et al., 2017), we observed no difference between lifting heavier *versus* lighter loads on RET-induced hypertrophy and strength when RET was performed to volitional fatigue (Fig. 2 and Table 3). A key discovery from this study was that RET-induced muscle hypertrophy was far less variable within an individual (i.e. independent of external load and limb location) than between individuals (Figs 2 and 4). This finding is important as we tested two widely disparate loading schemes (HL and LL) in two muscle groups. Nonetheless, the hypertrophic response, which we measured using multiple methods (Fig. 2 and Table 3), remained relatively conserved as did strength (Fig. 4), suggesting

that individuals who are higher responders to RET in the upper body would also exhibit similar results in the lower body, and vice versa. These data go some way towards addressing a persistent issue: whether response variability is conserved. Fractional muscle

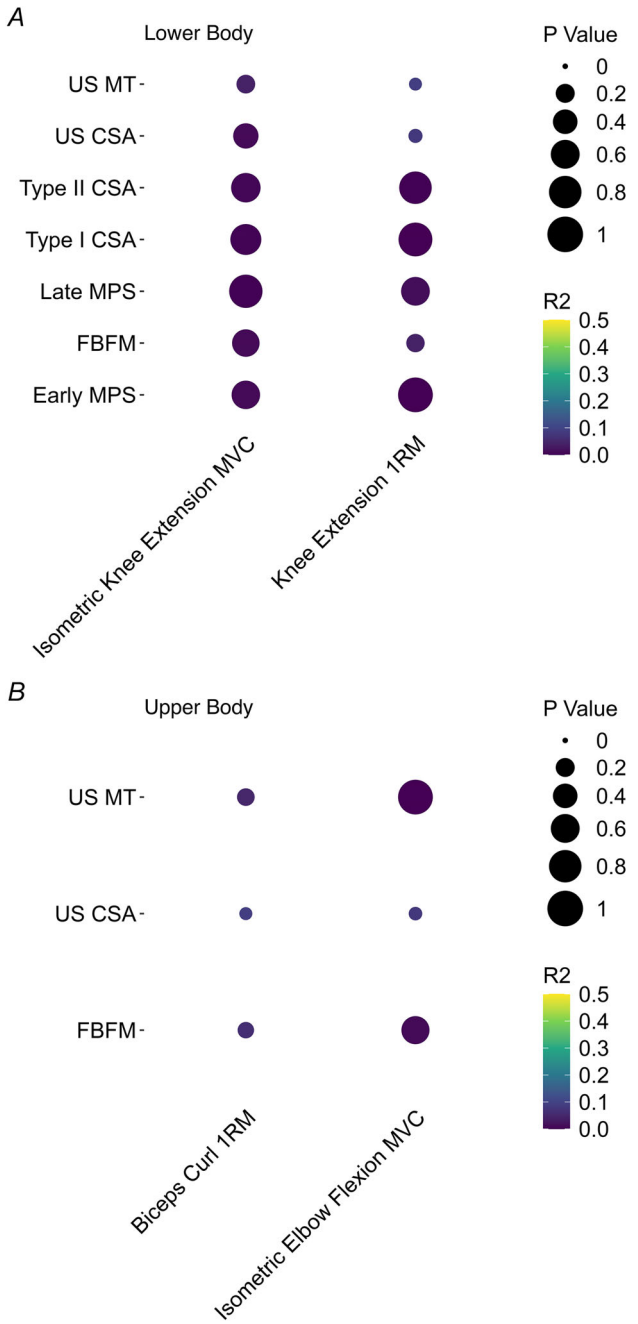

Figure 3. Dot plot depicting the association between baseline isometric and 1RM strength and changes in measures of skeletal muscle hypertrophy
(*A*) for the lower body and (*B*) for the upper body. The colour of the dot corresponds to the Pearson coefficient of determination (R2; R^2), and the size of the dot is proportional to the *P*-value.

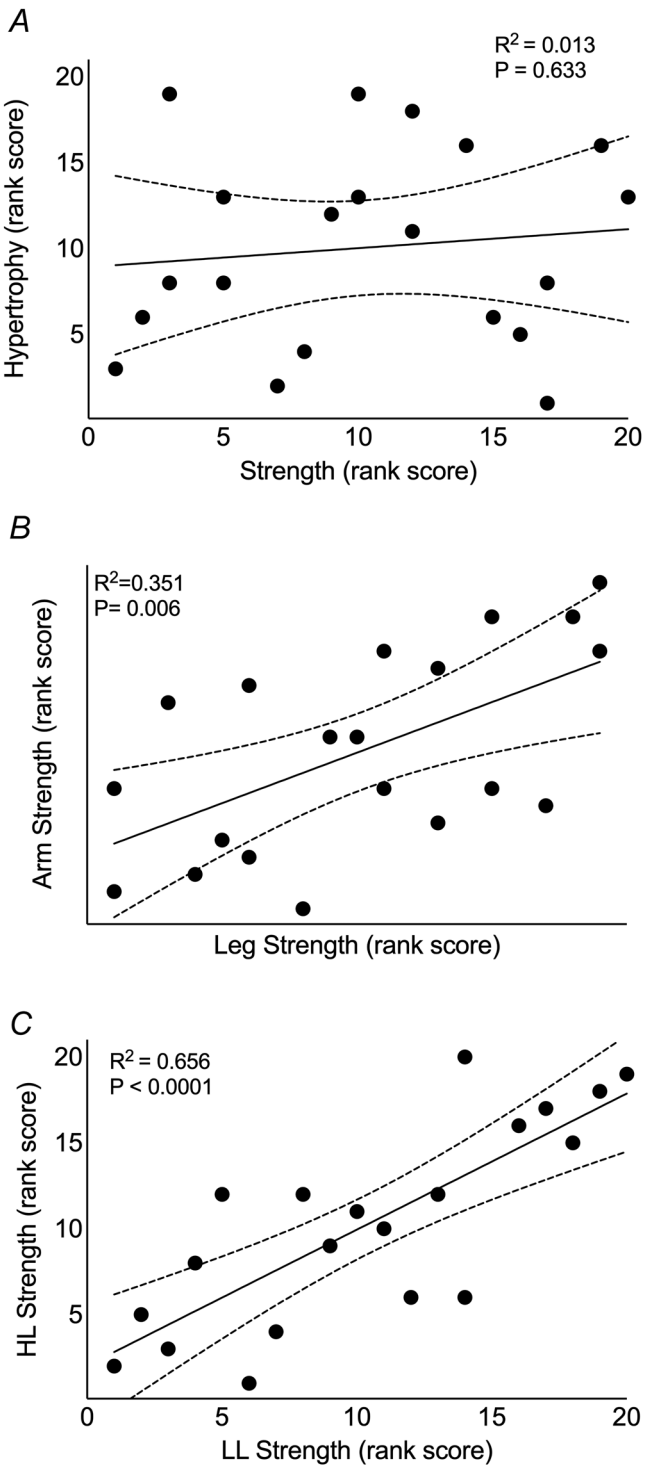

Figure 4. Ordinary least squares regression showing within-person shared variance
(*A*) between the rank score of hypertrophy and strength, (*B*) between strength at the upper- and lower body and (*C*) between lower-load (LL) and higher-load (HL) conditions. Each individual is represented by a single dot, and the dotted lines indicate the 95% confidence intervals.

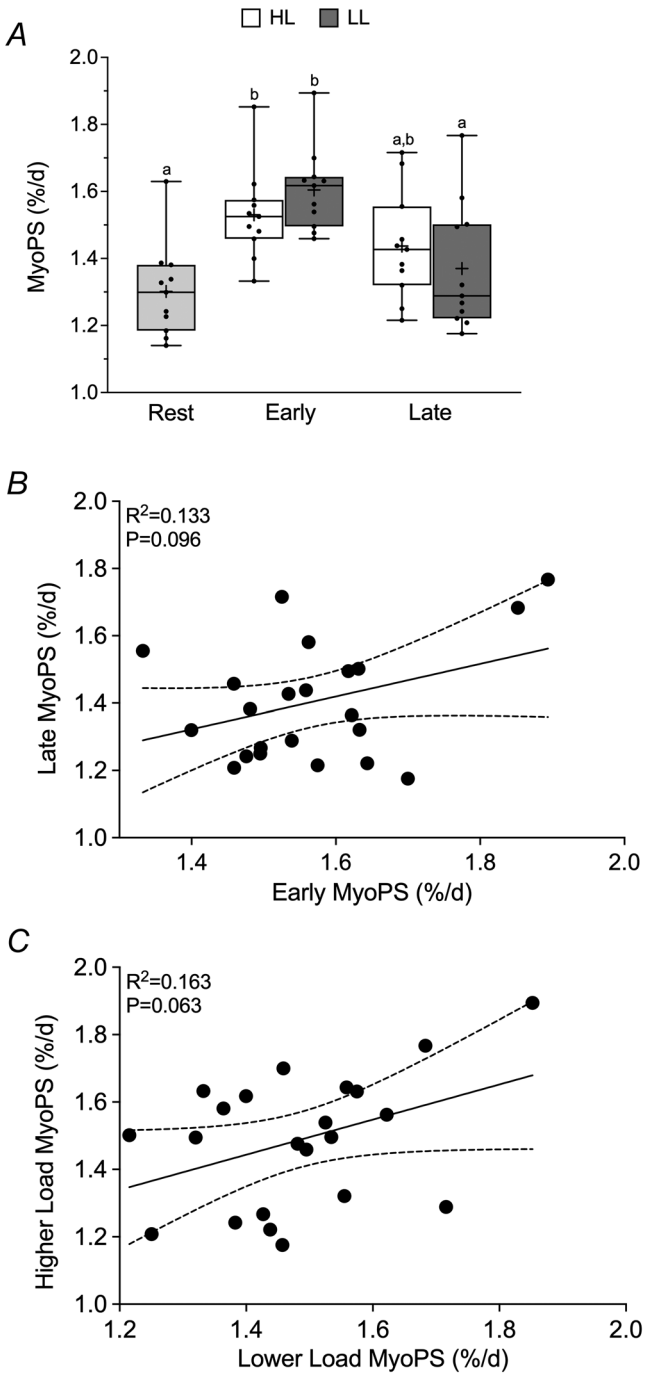

Figure 5. Resistance exercise-induced increases in integrated myofibrillar protein synthesis rates in the vastus lateralis are attenuated with training and are independent of load
A, integrated myofibrillar protein synthesis rates (MyoPS) before resistance exercise training (RET) (Rest; Week 0), during the first week of RET (Early; Week 1) and the last week of RET (Late; Week 10). Shared variation (correlation) in integrated MyoPS rates by RET duration (*B*) and load (*C*). Conditions that do not share a superscript letter are significantly different. Two-way repeated-measures ANOVA with time and condition as the within-subject variables was used. In the presence of significant main and/or interaction effects Tukey's honestly significant difference (HSD) *post hoc* tests were used to assess the differences between means.

protein synthesis rates were also more consistent within an individual (independent of load and RET duration) and were reduced with training experience (Fig. 5), which concurs with what we and others have shown previously (Brook et al., 2015; Damas et al., 2016; Kim et al., 2005; Phillips et al., 2002). Collectively, our data show that resistance exercise training adaptations are largely conserved within individuals, independent of exercise training load.

We observed that neither load nor limb location had a measurable effect on RET-induced muscular hypertrophy (Fig. 2). Additionally, there was significant shared variance (and lower CV) in RET-induced changes in muscle size and strength within individuals compared to between individuals (Figs 2 and 4; Table 2). Our data broadly align with other work (Angleri et al., 2022) and meta-analyses (Currier et al., 2023; Lopez et al., 2021; Schoenfeld et al., 2019), suggesting that although statistically significant differences in hypertrophy may be observed with RET variable manipulation, the effect sizes are generally small. Thus, manipulating external load has limited effects on RET-induced muscle hypertrophy when RET is performed to achieve, or at least approach, volitional fatigue (Morton et al., 2019). Similarly, the lack of difference between loading conditions in the unpractised strength task using isometric peak torque is similar to what has been reported in previous RET studies (Cholewa et al., 2018; Counts et al., 2016; Fisher & Steele, 2017; Mattocks et al., 2017; Schoenfeld et al., 2017; Jessee et al., 2018). There is now substantial evidence that heavier loads, although sufficient, are not necessary for RET-induced increases in muscle hypertrophy and are only marginally superior (with small effect sizes) for strength development (Carvalho et al., 2022; Currier et al., 2023), which is largely determined by practice. Because those lifting heavier loads during RET consistently practise lifting loads that are closer to the loads they will lift in a maximal isotonic strength test (i.e. the test used to assess 'strength gains'), it is hardly surprising that they perform better in a 1RM test (Morton et al., 2019). Notably, when participants lift lighter and heavier loads but are given opportunities to practise their 1RM, the strength differences between lighter and heavier training schemes are essentially null (Morton et al., 2016).

We observed elevated MyoPS rates in the early phase of RET compared to rest and an attenuated response at the late phase of RET despite progressive overload. Importantly, there was no difference in MyoPS rates between HL and LL conditions at any time. These observations align with prior work, suggesting that integrated MyoPS rates are initially stimulated to a greater degree, possibly due to greater exercise-induced muscle damage and are subsequently attenuated as muscle damage is reduced over time. After 10 weeks integrated MyoPS rates are closely associated with skeletal muscle

hypertrophy (Damas et al., 2016). The lack of observed differences in MyoPS rates between HL and LL conditions, and the fact that MyoPS rates were relatively homogenous within (i.e. HL leg *versus* LL leg) *versus* between individuals, is consistent with the findings of Damas et al. (2019), who noted that between-subject variability was 40-fold greater than that promoted by the extrinsic manipulation of RET variables (load, volume, contraction type and rest). Taken together, the findings presented here suggest that manipulation of an exogenous variable (i.e. resistance training load) modestly contributes to the resistance exercise-induced muscle protein synthetic response.

In terms of limitations the use of unilateral designs in exercise physiology studies can improve statistical power (MacInnis et al., 2017); however, a common critique of such designs is the cross-limb education effect (Manca et al., 2021). Indeed, a meta-analysis concluded that unilateral strength training increases both isotonic and isometric strength of the contralateral non-exercising limb (Manca et al., 2017), so it is plausible that RET-induced changes in muscle strength between HL and LL limbs were closer than those that may have been observed using a between-group design. In contrast, there is scant evidence that unilateral RET results in hypertrophy of the contralateral, untrained limb (Hubal et al., 2005; Wilkinson et al., 2006). Furthermore postexercise protein turnover rates are specific to the contracting muscle group (Holwerda et al., 2018; Wilkinson et al., 2014). We acknowledge that the implementation of muscle biopsy sampling from the BB in the present study would have enabled the determination of limb-specific muscle fibre CSA and MyoPS rates following HL and LL, as well as comparisons with VL-derived measures. Recently, it has been suggested that myofibre shape could be a hallmark of skeletal muscle health, and the extent of myofibre deformity could be reversed by resistance exercise training (Soendenbroe et al., 2024). A limitation of the current study is that we set a circularity cut-off of >0.80, thus excluding irregularly shaped myofibres from the fibre CSA analyses.

As others have reported (Haun et al., 2019) we observed very few correlations among measures commonly used to estimate hypertrophy. Furthermore, we demonstrate that indices of RET-induced increases in muscle size are unrelated to RET-induced increases in muscle strength (Figs 3 and 4*A*), contrary to our initial hypothesis (Erskine, Fletcher et al., 2014) but consistent with evidence from others (Loenneke et al., 2019). We observed minimal correlations between different indices of muscle size and strength. The significant negative correlation we observed between type I and type II fibre CSA and US CSA in the VL is consistent with prior research that used comparable training periods (Ruple, Mesquita et al., 2022; Ruple, Smith et al., 2022). Additionally, relative to other measurements reported in the current study we observed large within-participant CVs for fibre CSA measurements (Table 2), a consistent finding with previous research (Ruple, Mesquita et al., 2022; Ruple, Smith et al., 2022). Collectively, the relatively poorer reliability with fibre CSA measurements should be taken into consideration when comparing fibre CSA to other macro-measures of skeletal muscle size. Thus,, in agreement with others (Haun et al., 2019), we strongly advocate using multiple measures of muscle size and strength for a comprehensive understanding of RET-induced adaptations, as illustrated here (Fig. 2).

We observed considerable interindividual variability in RET-induced increases in hypertrophy indices and strength over 10 weeks of RET. However, both RET-induced increases in muscle size and strength occurred independently of load and limb location and were relatively consistent within an individual. Our finding of little influence of external load on RET-induced hypertrophy is consistent with the reported small contribution of other exogenous variables to the RET-induced hypertrophic response. We conclude that when momentary muscular fatigue (or approaching fatigue) is used as an end-point for RET or as a proxy for a high degree of effort (i.e. both higher and lower loads were lifted to the point of momentary muscular fatigue), neither load nor limb location mediates RET-induced hypertrophy. It is plausible to suggest that interindividual variability in RET-induced muscular hypertrophy is primarily mediated by changes in endogenous variables (Lim et al., 2022), but further work is warranted.

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Additional information

Data availability statement

Data are available upon reasonable request from the corresponding author.

Competing interests

S.M.P. reports grants or research contracts from the US National Dairy Council, Dairy Farmers of Canada, Roquette Freres, Nestle Health Sciences, Myos, National Science and Engineering Research Council, Canadian Institutes for Health Research and the US NIH during the conduct of the study; personal fees from US National Dairy Council, non-financial support from Enhanced Recovery, outside the submitted work. S.M.P. has a patent (Canadian) assigned to Exerkine and a patent (USA) pending to Exerkine, but reports no financial gains from any patent or related work. The remaining authors report no competing interests.

Author contributions

R.W.M. and S.M.P. designed the study; R.W.M., M.D.F., S.R.M., R.S.S., C.M. and S.M.P. performed the data collection; M.J.L., J.C.M., R.W.M., M.D.F., B.J.C., S.R.M., R.S.S., B.N.S., E.K.W., J.C.M. and S.M.P. performed the data analysis; M.J.L., J.C.M., R.W.M. and S.M.P. drafted the manuscript; and all authors critically revised the manuscript and gave their approval to the final version. All authors have approved the final version of the manuscript submitted for publication and agreed to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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