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ORIGINAL ARTICLE

Individual muscle hypertrophy response is affected by the overload progression model and is associated with changes in satellite cell content

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Abstract

Purpose We aimed to compare individual hypertrophic responses to resistance training in which overload progressed either by adjusting the load (LOADProg) or by increasing the number of repetitions (REPSProg). Furthermore, we investigated whether greater responsiveness to one protocol was associated with chronic changes in myonuclei and satellite cells, proteolysis and extracellular matrix (ECM) remodeling biomarkers.

Methods Thirty-seven untrained participants had their legs randomized into LOADProg and REPSProg and underwent 10 weeks of training. Muscle cross-sectional area (mCSA) ultrasound and muscle biopsies were performed pre- and post-training. Based on mCSA changes between protocols, we applied a criterion of 2 typical errors (5.7%) to create 4 clusters.

Results Twelve participants (~34%) showed greater mCSA increases after REPSProg (14.2 ± 7.6%) than LOADProg (3.4 ± 8.7%, $p = 0.004$). Seven participants (~19%) responded better to LOADProg (21.5 ± 7.5% vs. 12 ± 7.5%, $p = 0.041$). Thirteen participants (~35%) showed no differences between protocols ($p = 0.852$). Five participants were nonresponders (mCSA changes smaller than the 5.7% threshold) for both protocols. There were no significant differences ($p > 0.05$) in myonuclear content, proteolysis, or ECM remodeling markers within any of the clusters. However, for those who responded better to REPSProg, this protocol promoted greater satellite cell changes (108.6 ± 77.0%) than LOADProg (48.9 ± 63.1%, $p = 0.015$).

Conclusion Our findings suggest that overload progression models may influence individual responsiveness to RT-induced muscle hypertrophy. Additionally, progression through increased repetitions was associated with a chronic addition of satellite cells. However, responsiveness was not explained by chronic changes in myonuclei, proteolysis or ECM remodeling biomarkers.

Trial registration This study is registered in the Brazilian Registry of Clinical Trials (RBR-57v9mrb).

Keywords Muscle cross-sectional area · Resistance training · Responsiveness · Satellite cells · Proteolysis

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Abbreviations

ANCOVA	Analysis of covariance
BMI	Body mass index

BSA	Bovine serum albumin
CI	Confidence interval
DAPI	4′,6-Diamidino-2-phenylindole
ECM	Extracellular matrix
ES	Effect size
fCSA	Fiber cross-sectional area
LOADprog	Load progression
MMPs	Matrix metalloproteinases
mCSA	Muscle cross-sectional area
OCT	Optimal cutting temperature
PBS	Phosphate-buffered saline
REPSprog	Repetitions progression
RFU	Relative fluorescence units
RLU	Relative luminescence units
RT	Resistance training
TBST	Tris-buffered saline and tween-20
TE	Typical error
TIMPs	Tissue inhibitors of metalloproteinases
$\chi 2$	Chi-square test
1RM	One repetition maximum

Introduction

Resistance training (RT) has been well established as an effective non-pharmacological intervention to promote muscle hypertrophy (Roberts et al. 2023). To ensure that hypertrophy occurs continuously, it is recommended to gradually progress the overload imposed by RT (Kraemer and Ratamess 2004). Conventionally, this progression is performed by adjusting the load within a repetition maximum zone (LOADprog) (i.e., load is adjusted set by set to ensure that concentric muscle failure occurs within the targeted repetition zone). Nonetheless, we recently reported that LOADprog promotes muscle gains similar to those observed when increasing the number of repetitions, (i.e., individuals perform as many repetitions as possible until muscle failure with a fixed load (REPSprog) (Chaves et al. 2024). However, some individuals may present greater muscle gains in response to one RT protocol than to another (e.g., REPSProg vs. LOADProg). Through a within-subject design in which each leg of the same participant is allocated to a different protocol, studies revealed that some individuals have greater muscle mass gains when training at high weekly frequency (Damas et al. 2019) or higher RT volume (Lixandrão et al. 2024), while others benefit more from a low frequency or lower volume. Hence, it is reasonable to suggest that different overload progression protocols could result in distinct individual responses, with some participants benefiting more from one protocol than from the other.

The difference in muscle mass gains between protocols performed by the same subject suggests a participant-by-training interaction that distinctly modulates the molecular processes regulating muscle hypertrophy. Among these processes, satellite cell activation has received considerable attention from the scientific community (Snijders et al. 2015; Murach et al. 2018; Roberts et al. 2023; Saliu et al. 2024). It is believed that resident myonuclei in myocytes have a limited transcriptional capacity, eventually reaching a ceiling effect. Therefore, to maintain hypertrophy capacity, it would be required the addition of new myonuclei, which are provided by the fusion of differentiated satellite cells into growing muscle fibers (Bamman et al. 2018). Additionally, several associations have been observed between RT responsiveness and satellite cell content or satellite cell-mediated myonuclear accretion (Petrella et al. 2008; Bellamy et al. 2014; Angleri et al. 2022a; Smith et al. 2023). Thus, it is plausible that if overload progression protocols promote distinct individual responses, the abundance of satellite cells and myonuclei may also be differentially modulated.

Other mechanisms have been indicated to be involved in muscle hypertrophy, but little is known about their contributions to individual hypertrophic responses (Roberts et al. 2018). For instance, the structural remodeling of skeletal muscles involves the activity of proteolytic systems that promote the recycling of damaged or inefficient cellular components (Pasiakos and Carbone 2014). Muscle remodeling involves changes not only in myocytes but also in the extracellular matrix (ECM). The ECM participates in several molecular processes, particularly as a repository for growth factors (Naba et al. 2016), facilitator in mechanical sensing and force transmission (Kjaer 2004), and is fundamentally involved in skeletal muscle regeneration (Mackey and Kjaer 2017). Evidence suggests that remodeling factors (e.g., matrix metalloproteinases [MMPs] and tissue inhibitors of metalloproteinases [TIMPs]) act to balance the ECM content, which can either be reduced to allow for myofiber hypertrophy or increased to strengthen the cellular scaffold (Roberts et al. 2023). However, none of these mechanisms have been explored regarding individual responsiveness to different RT protocols.

Therefore, the present study aimed to compare individual muscle hypertrophy in response to REPSProg and LOADProg. For this, we used data from our previous group analysis study that assessed young untrained participants in a within-subject design (Chaves et al. 2024). Additionally, we sought to explore whether protocol responsiveness could be accompanied by training-induced changes in satellite cell and myonuclei counts per muscle fiber, as well as in the protein content and enzymatic activity levels of proteolytic and ECM remodeling markers following 10 weeks of RT (i.e., chronic changes).

Methods

Participants

This is an ancillary analysis of data and muscle samples derived from a randomized controlled trial (Brazilian Registry of Clinical Trials—RBR-57v9mrb). The study was approved by the local ethics committee and conducted in accordance with the most recent version of the Declaration of Helsinki. Following the interviews and screening, 53 recreationally active young women and men were selected from the community to participate. The inclusion criteria were as follows: 18 to 35 years of age and not engaged in any exercise training program for at least 6 months prior to the study. Exclusion criteria involved the use of any drugs that could affect skeletal muscle mass or strength and any musculoskeletal disorders or injuries that could contraindicate RT practice. After being informed about the experimental procedures and potential associated risks and benefits, all participants signed a written consent form agreeing to enroll in the study. Eleven participants reported significant discomfort after the first muscle biopsy and chose to withdraw from the study. It is important to note that these individuals were not engaged in regular exercise prior to the study and, therefore, were likely less accustomed to experiencing physical discomfort or pain. Additionally, because participants were informed that multiple biopsies would be required throughout the study, those who found the first procedure particularly uncomfortable may have opted to withdraw rather than undergo additional procedures. Four participants withdrew during the experimental period for personal reasons unrelated to the intervention. One participant did not complete post-intervention muscle biopsies and was therefore excluded from this analysis. Thus, the final sample of this study included 37 participants: eighteen women (age: 23.8 ± 4.6 years; body mass: 61.7 ± 7.7 kg; height: 163 ± 4.8 cm; BMI: 23.3 ± 3.3 kg/m^2) and nineteen men (age: 24.5 ± 3.5 years; body mass: 73.0 ± 13.9 kg; height: 176 ± 6.6 cm; BMI: 23.4 ± 4.2 kg/m^2).

Experimental design

This was a within-subject study with repeated measures over time. Initially, participants had the muscle cross-sectional area of their vastus lateralis acquired via ultrasound at two different visits, with a 72-h interval in between, to calculate the reproducibility of measurements. Next, a one repetition maximum (1RM) test was performed as described previously by our group (Scarpelli et al. 2022). For each participant, the right and left legs were randomly allocated to one of two different RT protocols. The randomization and allocation processes were based on leg dominance, leg strength (stronger/weaker) and the degree of strength difference between legs (high/moderate/low). Over the following 10 weeks, participants engaged in supervised RT sessions, 2–3 times a week, totaling 23 training sessions. Both RT protocols were performed in the same session in a randomized and counterbalanced order. Further details on the randomization and allocation processes are described in Chaves et al. (2024). Muscle samples were collected via muscle biopsies from each leg immediately before the first RT session. The ultrasound assessments and muscle biopsies were repeated 96 h after the last (23rd) RT session.

Training protocols

LOADprog consisted of 4 sets of 9–12 repetitions maximum of the unilateral leg extension exercise. The initial load of 80% of the 1RM. The initial load was set at 80% of 1RM. The load was adjusted on a set-by-set basis when concentric muscle failure occurred outside the target repetition range. Therefore, overload progressed through increases in load (LOADprog). The REPSprog was performed on the contralateral leg and consisted of 4 sets of unilateral leg extension exercises performed until concentric muscle failure with a fixed load equivalent to 80% of the initial 1RM. The load was not adjusted during the experimental period; therefore, overload progression was achieved by increments in the number of repetitions (REPSprog) whenever the participant was able to postpone muscle failure. Participants were verbally encouraged throughout both protocols, and a 90-s rest interval was allowed between sets. To mitigate potential crossover effects between protocols, the sequence of execution was randomized and counterbalanced across training sessions, as previously described in our prior study (Chaves et al. 2024). Specifically, participants alternated the order in which each leg performed the assigned training protocol to minimize systematic biases. Additionally, a standardized rest period of 2 min was implemented between protocols within the same session.

For each protocol, the load (kg) and the number of repetitions were recorded for each set at each training session. The sum of repetitions performed throughout the intervention period was used to obtain the average number of repetitions per set per session. The sum of loads utilized throughout the intervention period was calculated to obtain the average load per set per session. The accumulated volume load was calculated as sets × repetitions × load (kg) considering the entire training period.

Muscle cross-sectional area (mCSA)

Participants were instructed to abstain from vigorous physical activity for at least 72 h before each assessment in order to minimize post-exercise edema (Damas et al. 2016). After arriving at the laboratory, the distance between the greater trochanter and the lateral epicondyle of the participants was measured, and the point corresponding to 50% of the femur length was marked as a reference for image acquisition. From this point, successive markings were made transversally, every 2 cm, in both the medial and lateral directions. The participant then laid still in a supine position for 15 min to allow tissue fluid stabilization. Sequential B-mode US images were acquired using a 7.5 MHz linear probe (MySono U6, Samsung, Sao Paulo, Brazil) and a water-soluble transmission gel to ensure acoustic coupling with minimal skin compression. Skin markings were used as a guide for the displacement of the probe in the sagittal plane, starting at the most medial mark (over the rectus femoris muscle). Using PowerPoint software (Microsoft, USA), the captured images were rotated and arranged to obtain a reconstructed panoramic image of the CSA of the entire muscle. The image was then opened on ImageJ (Rasband 2011), and the "polygon" tool was used to trace and calculate vastus lateralis CSA, excluding connective and bone tissue from the delimited area. The typical error between two image acquisitions and quantifications separated by 72 h was 0.60 cm^2 (2.85%).

Muscle biopsies

Samples of the vastus lateralis muscle were obtained through a percutaneous muscle biopsy performed by a qualified medical professional with extensive experience in the procedure. Before tissue extraction, the region was cleaned with an antiseptic wash, and local subcutaneous anesthesia was applied [2–3 ml of 1% xylocaine (lidocaine)]. A small incision was made with a surgical scalpel, and a biopsy needle (Bergström model with manual suction) was introduced to a depth of approximately 4 cm to remove ~ 100 mg of muscle tissue, which was cleaned of all blood and connective tissue and separated into aliquots for analysis. The incision was closed and covered with bandages. A portion of tissue (20–30 mg) was positioned in liquid embedding medium for optimal cutting temperature (OCT), with the fibers perpendicular to the horizontal surface, and quickly frozen in nitrogen-cooled isopentane for immunohistochemical analyses. The remaining portion was placed in prelabeled RNA-free cryotubes for biochemical analyses. After separation, all the samples were immediately frozen in liquid nitrogen and stored at -80°C until processing.

Tissue homogenization

The neat muscle tissue was weighed using an analytical scale (Mettler Toledo ME303, Greifensee, Switzerland), and approximately 20 mg were placed in 1.7 ml microcentrifuge tubes. The samples were then homogenized in 500 µl of cell lysis buffer (cat. no. 9803; Cell Signaling Technology, Danvers, MA, USA) using hard plastic pestles and subsequently centrifuged at 500 × g for 5 min at 4 °C. The resulting supernatants were transferred and stored in a new set of microtubes. Total protein concentrations were then determined using a bicinchoninic acid (BCA) protein assay kit (cat. no. 23227; Thermo Fisher Scientific) and a spectrophotometer (BioTek Synergy H1, Winooski, VT, USA) following the manufacturer's instructions.

Western blotting

The cell lysates were added to 4 × Laemmli buffer and distilled water (diH2O) to reach a 1 µg/µl protein concentration. Samples were then denatured for 5 min at 100 °C prior to being stored at −80 °C until analysis. Thereafter, the prepared samples (15 µl) were loaded onto the wells of 4−15% SDS−polyacrylamide gels (cat. no. 5671085, Criterion TGX; Bio-Rad Laboratories, Hercules, CA, USA) and subjected to electrophoresis at 180 V for 50 min with premade 1 × SDS−PAGE running buffer (cat. no. RLMB-017, VWR-Avantor, Radnor, PA, USA). Proteins were then transferred to preactivated polyvinylidene difluoride membranes (cat. no. 1620177; Bio-Rad Laboratories) at 200 mA for two hours. Next, the membranes were stained with Ponceau for 10 min and digitally imaged with a gel documentation system (ChemiDoc Touch; Bio-Rad Laboratories). Next, the membranes were reactivated in methanol and blocked for one hour in a solution of 5% nonfat milk powder in Tris-buffered saline and 0.1% Tween-20 (TBST). The membranes were then incubated overnight at 4 °C in an antibody solution containing a 1:1000 dilution in TBST supplemented with 5% bovine serum albumin (BSA) and one of the following: (i) rabbit anti-calpain-1 (cat. no.: 2556; Cell Signaling Technology); (ii) rabbit anti-calpain-2 (cat. no.: 70655; Cell Signaling Technology); (iii) rabbit anti-ubiquitin (cat. no.: 3933; Cell Signaling Technology), (iv) rabbit anti-20S core subunit antibody cocktail (cat. no. BML-PW8155; Enzo Life Sciences; Farmingdale, NY, USA); (v) rabbit anti-p62/SQSTM1 (cat. no. 5114; Cell Signaling Technology); (vi) rabbit anti-human MMP-9 (cat. no. ab76003; Abcam, Cambridge, MA, USA); (vii) rabbit anti-human MT1-MMP/MMP-14 (cat. no. 13130; Cell Signaling Technology); (viii) rabbit anti-human TIMP-1 (cat. no. 8946; Cell Signaling Technology); (ix) rabbit anti-human TIMP-2 (cat. no. 5738; Cell Signaling Technology). The next day, the membranes were incubated for one hour with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (cat. nos. 7076 and 7074; Cell Signaling Technology). The membranes were then developed using chemiluminescent substrate (Immobilon Forte, cat. no. WBLUF0500; MilliporeSigma, Burlington, MA, USA) and digitally imaged using a gel documentation system (ChemiDoc Touch; Bio-Rad Laboratories) and densitometry associated software (Image Lab v6.0.1; Bio-Rad Laboratories). Densitometry values were normalized to Ponceau densities.

Enzymatic activity assays

Calpain and proteasome activities on lysates were assayed using commercially available luminescence kits (cat. no.: G8502 and cat. no.: G8622; Promega Corporation, Madison, WI, USA). Briefly, 25 µl of lysates were loaded onto white 96-well plates in duplicate and incubated at room temperature for 10 min with the reagent mix provided by each kit. For global MMP activity, a commercially available fluorometric assay kit was used (cat. no.: AS-72202; AnaSpec, Freemont, CA, USA). Muscle tissue lysates (50 µl) were loaded in duplicate into black 96-well microplates with the substrate solution provided by the kit and incubated at room temperature for 30 min. Luminescence and fluorescence readings were performed with a microplate luminometer (BioTek Synergy H1, Winooski, VT, USA), and the values were divided by the total protein loaded per well and are expressed as relative luminescence/fluorescence units (RLU; RFU) per µg of muscle soluble protein. The fluorescence intensity was read using 330 nm excitation and 390 nm emission settings. The average coefficients of variation for duplicate values of calpain, proteasome and MMP activity were 5.2%, 5.4% and 6.5%, respectively.

Immunohistochemical analyses

OCT-preserved samples were transferred to a cryostat (Leica Biosystems, Buffalo Grove, IL, USA) set at −23 °C, sectioned at a thickness of 14 µm, adhered to positively charged histology slides and stored at −80 °C until batch-processed for immunohistochemical analyses. Samples from the same participant were placed on the same slide and analyzed concomitantly.

After being air-dried at room temperature for two hours, the slides were fixed with acetone at −20 °C for 5 min. Slides were incubated with 3% H_2O_2 for 10 min at room temperature, followed by a 1-min incubation with autofluorescence quenching reagent (TrueBlack, cat. no. 23007; Biotium, Fremont, CA, USA). Next, the slides were blocked for 60 min with 5% goat serum, 2.5% horse serum, and 0.1% Triton-X solution at room temperature. Subsequently, the slides were blocked with streptavidin and biotin solutions (cat. no. SP2002; Vector Laboratories, Newark, CA, USA) at room temperature for 15 min each. After blocking, the slides were incubated overnight at 4°C with a primary antibody cocktail of 1:20 Pax-7 (cat. no. pax7; RRID: AB_528428; Developmental Studies Hybridoma Bank; Iowa City, IA, USA), 1:100 Dystrophin (cat. no. GTX57970; GeneTex, Irvine, CA, USA), 1:100 BA-D5 (Myosin Heavy Chain I) (cat. no. BA-D5; RRID: AB_2235587; Developmental Studies Hybridoma Bank) and 2.5% horse serum in phosphate-buffered saline (PBS). The next day, the slides were incubated for 60 min with a 1:1000 solution of biotin-conjugated anti-mouse IgG1 (cat. no. 115-065-205; RRID: AB_2338571; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) in 2.5% horse serum, followed by a 60-min incubation with a secondary antibody cocktail of 1:500 streptavidin (SA-HRP, cat. no. S911; Thermo Fisher Scientific, Waltham, MA, USA), 1:250 goat anti-mouse IgG2b Alexa Fluor 647 (cat. no. A-21242; Thermo Fisher Scientific), 1:250 goat anti-rabbit IgG DyLight488 (cat. no. DI-1488; Vector Laboratories) in PBS, and a 20-min incubation with 1:200 TSA-555 (cat. no. B40955; Thermo Fisher Scientific). All steps mentioned above involved gentle washes in PBS. Slides were then stained with DAPI (4 = ,6-diamidino-2-phenylindole; cat. no. D3571; Thermo Fisher Scientific) for 10 min at room temperature and mounted with glass coverslips using 1:1 PBS and glycerol as mounting medium. The sections were stored in the dark at 4°C until imaging. Digital images were captured using a fluorescence microscope set at × 20 magnification (Nikon Instruments; Melville, NY, USA). An average of 230 fibers per sample were quantified (Mackey et al. 2009), and all areas selected for analyses were free of freeze-fracture artifacts. The staining method allowed the identification of cell membranes (detected by the TRITC filter), type I fiber cell bodies (detected by the FITC filter), type II fiber cell bodies (unlabeled), satellite cells (detected by the Texas Red filter), and myonuclei (detected by the DAPI filter).

Standardized counting of muscle fibers and myonuclei was performed via open-sourced software (MyoVision) (Wen et al. 2018), applying a conversion ratio of 0.46 µm/pixel and a detection range from 500 to 15,000 µm^2. Next, an investigator visually verified the software results, and empty spaces or incorrectly outlined fractured fibers were manually excluded from the analysis. The number of satellite cells on the laminin border was determined via visual inspection and verification of the colocalization of Pax7 + and DAPI. For each sample, the content of myonuclei and satellite cells was normalized to the respective number of muscle fibers analyzed. Notably, all procedures—sectioning, staining, imaging—and analyses were performed by investigators who were blinded to the interventions.

Statistical analyses

Participants were separated into four clusters based on their hypertrophic responses, and 2 typical errors (2 × TE) of mCSA measurements were used as the cutoff criterion. A change in mCSA greater than this threshold would have a high probability of being a true different adaptation beyond any technical and/or biological variability (Hopkins 2001). Specifically, the TE was calculated using the following equation:

$$TE = \frac{SD_{diff}}{\sqrt{2}}$$

where SD_{diff} is the standard deviation (SD) of the difference between the two baseline mCSA measurements obtained through the US assessments. The TE of the present sample was 0.60 cm^2, yielding a 2 × TE value of 1.2 cm^2. We used the 2 × TE criterion, expressed as a percentage of the mean of the baseline mCSA measurements (Hopkins 2001), to compare it to the relative pre-to-post intervention changes in mCSA. Hence, our clustering criterion was 5.7%.

Figure 1 presents a flowchart summarizing the clustering procedure. First, a participant was defined as a "responder" to RT if she or he presented muscle gains greater than 2 × TE for at least one of the RT protocols; otherwise, she or he was considered a "NONRESPONDER". Second, for "responders", we compared hypertrophy results between legs. If the difference between muscle gains was smaller than 2 × TE, the participant was classified as a "NO DIFFERENCE RESPONDER". If the difference was greater than 2 × TE, the participant was placed in either the "REPERS" or the "LOADERS" cluster, depending on whether greater gains were observed in response to REPSprog or LOADprog, respectively.

All statistical analyses were conducted for each cluster separately. Analyses of covariance (ANCOVA) were implemented for all outcomes to compare relative chronic changes (from Pre to Post) with training protocol as a fixed factor, Pre values as covariates, and participants as random factors. The normality of residuals and the effect of extreme observations were determined through residual analyses and influence diagnostics. Additionally, for each outcome, the effect size (ES) and the respective 95% confidence interval (CI) were calculated (Hedges and Olkin 1985) between relative changes observed in the REPSprog and LOADprog protocols. Positive and negative CIs not crossing zero (0) were considered significant (Nakagawa and Cuthill 2007). Repeated measures correlations were calculated (Bakdash and Marusich 2017) to determine the within-cluster

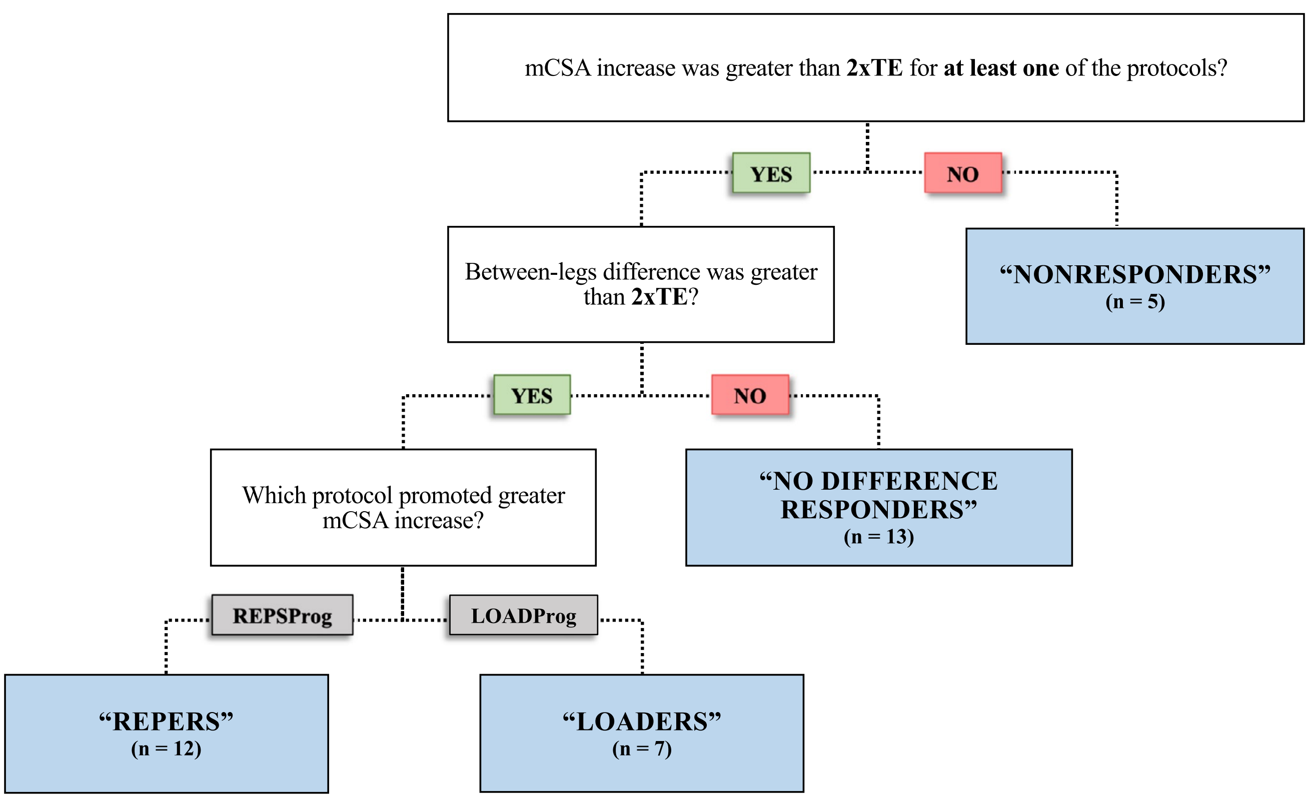


Fig. 1 Flowchart of the clustering process

associations between relative chronic changes in mCSA and relative chronic changes in the assayed markers. Data from the same participant following the two protocols were considered as paired measures. Correlations were classified as very weak ($|r| < 0.20$), weak ($0.20 < |r| < 0.39$), moderate ($0.40 < |r| < 0.59$), strong ($0.60 < |r| < 0.79$) or very strong ($|r| > 0.80$) (Evans 1996). Paired t tests were used to compare the accumulated volume load, the average number of repetitions, and the average load between protocols. A chi-square test of goodness-of-fit was performed to determine whether the observed frequency of REPERS and LOADERS was significantly different. All data are presented as mean ± standard deviation (SD), unless otherwise stated. Significance was set at $p < 0.05$. Analyses were performed using SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA), the rmcorrShiny application (Marusich and Bakdash 2021) and GraphPad Prism 9.5 software (GraphPad Software, San Diego, CA, USA). Graphs were constructed using GraphPad Prism 9.5.

Results

Loaders

Seven participants (3 women and 4 men) were deemed "LOADERS"; i.e., their response to RT met the 2 × TE criterion and muscle gains favored LOADProg in over 5.7% (2 × TE) compared to REPSProg. As per design, ANCOVA results showed that mCSA relative changes from pre- to post-intervention were lower for REPSProg (12 ± 7.5%) than for LOADProg (21.5 ± 7.5%%; $F = 70.5$, $p = 0.0004$) (Fig. 2). The CI of the ES (ES: -1.18; 95% CI: -2.31 to -0.04) confirmed the inferential analysis.

A t-test revealed no significant differences in volume load between LOADProg (47.4 ± 17.9 tons) and REPSProg (50.1 ± 18.8 tons; $p = 0.616$) (Fig. 2). The average number of repetitions performed per set per training session was greater for REPSProg (13.2 ± 3.1) than for LOADProg (10.4 ± 1.0; $p = 0.021$), with no significant differences in the average load utilized per set per training session (LOADProg: 48.6 ± 15.1 kg; REPSProg: 41.6 ± 14.8 kg; $p = 0.194$).

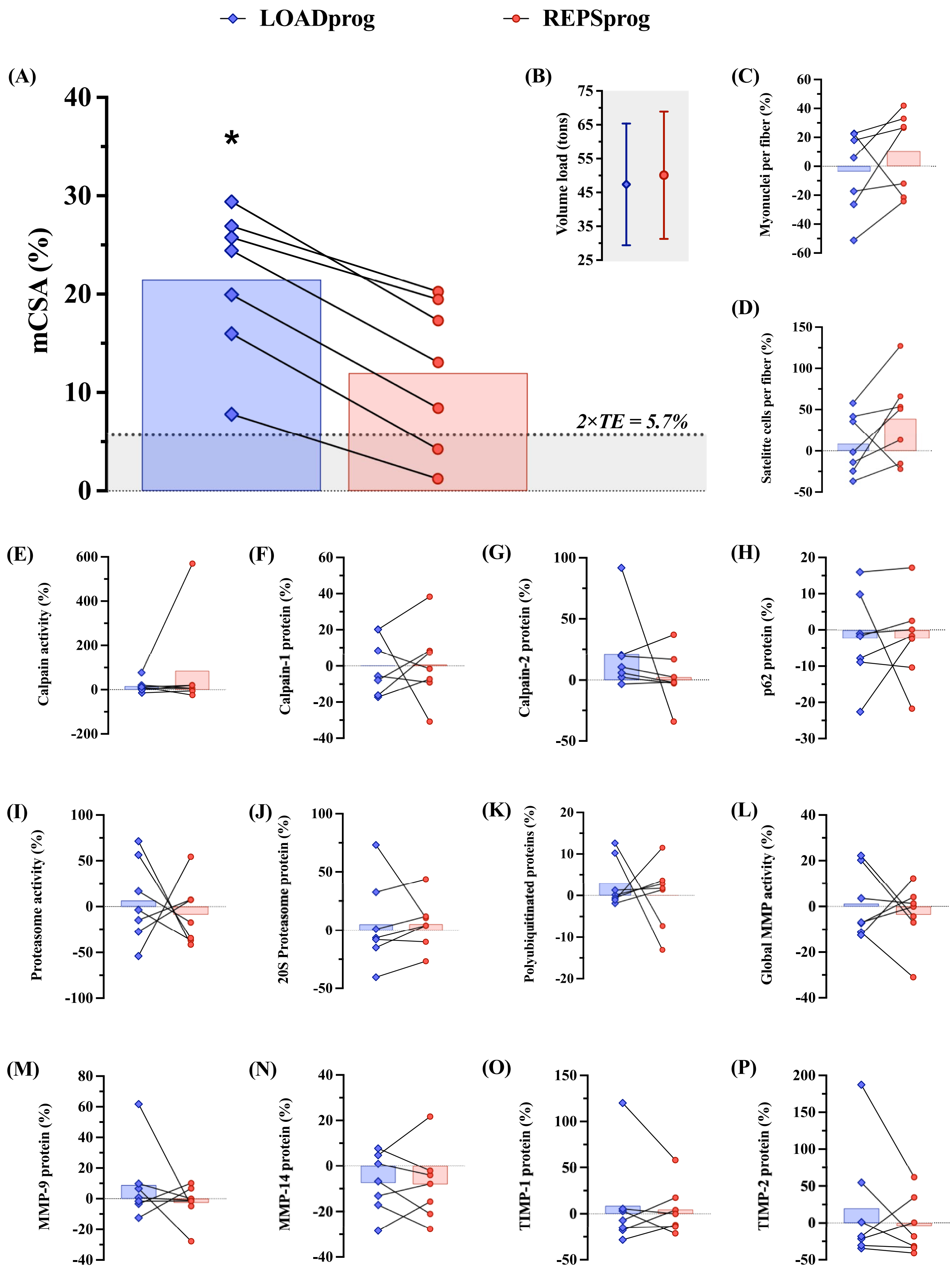
ANCOVA revealed no significant differences between protocols on any of the biomarkers investigated (Fig. 2): (i) myonuclei ($F = 0.52$, $p = 0.505$), (ii) satellite cell counts ($F = 2.26$, $p = 0.193$), (iii) global calpain activity ($F = 2.20$, $p = 0.198$), (iv) global proteasome activity ($F = 0.26$, $p = 0.631$), (v) global MMP activity ($F = 0.10$, $p = 0.769$), (vi) calpain-1 protein content ($F = 0.00$, $p = 0.956$), (vii) calpain-2 protein content ($F = 0.00$, $p = 0.967$), (viii) 20S proteasome protein content ($F = 0.51$, $p = 0.506$), (ix) polyubiquitinated proteins ($F = 0.32$, $p = 0.598$), (x) p62 ($F = 0.00$, $p = 0.990$), (xi) MMP-9 protein content ($F = -0.38$, $p = 0.717$), (xii) MMP-14 protein content ($F = 0.11$, $p = 0.756$), (xiii) TIMP-1 protein content ($F = 0.01$, $p = 0.937$), or (xiv) TIMP-2 protein content ($F = 0.27$, $p = 0.628$). The inferential analysis was confirmed by the CIs of the respective ESs. Repeated measures correlation analysis revealed no correlation between chronic changes in mCSA and chronic changes in the assayed markers. The relative changes (post vs. pre), the correlation coefficients and the ES and CI between protocols are presented in the supplemental material (Table 1).

Repers

A total of 12 participants (6 women and 6 men) were classified as "REPERS", i.e., the response to RT met the 2 × TE criterion, and muscle gains favored the REPSProg protocol, with a difference greater than 2 × TE compared to the LOADProg protocol. In accordance with our design, the ANCOVA results indicated that mCSA relative changes were greater for REPSProg (14.2 ± 7.6%) than for LOADProg (3.4 ± 8.7%; $F = 145.94$, $p < 0.0001$) (Fig. 3). The CI of the ES (ES: 1.27; 95% CI: 0.39 to 2.15) confirmed the inferential analysis.

A t-test showed no significant differences in the volume load between LOADProg (53.4 ± 18.1 tons) and REPSProg (53.1 ± 19.5 tons; $p = 0.829$) (Fig. 3). The average number of repetitions performed per set per training session was greater for REPSProg (14.0 ± 3.1) compared to LOADProg (10.7 ± 0.8; $p < 0.001$), while the average load utilized per set per training session was greater for LOADProg (55.3 ± 17.4 kg) than for REPSProg (41.8 ± 14.2 kg; $p < 0.001$).

For the biomarkers assessed (Fig. 3), results revealed that the relative chronic changes in satellite cell count per fiber were greater for REPSProg (108.6 ± 77.0%) than for LOADProg (48.9 ± 63.1%; $F = 9.04$, $p = 0.015$). As for the protein content of TIMP-1, chronic changes were lower for REPSProg (−6.1 ± 22.4) than for LOADProg (5.1 ± 18.2%; $F = 7.75$; $p = 0.019$). There were no significant differences in the other variables including: (i) the number of myonuclei ($F = 0.84$; $p = 0.381$), (ii) global calpain activity ($F = 0.49$; $p = 0.500$), (iii) global proteasome activity ($F = 0.13$, $p = 0.724$), (iv) global MMP activity ($F = 0.14$, $p = 0.719$), (v) calpain-1 protein content ($F = 0.04$, $p = 0.829$), (vi) calpain-2 protein content ($F = 0.19$, $p = 0.674$), (vii) 20S proteasome protein content ($F = 0.55$, $p = 0.474$), (viii) polyubiquitinated proteins ($F = 0.03$, $p = 0.861$), (ix) p62 protein content ($F = 1.08$, $p = 0.323$), (x) MMP-9 protein content ($F = 0.31$, $p = 0.589$), (xi) MMP-14 protein content ($F = 0.00$, $p = 0.970$), or (xii) TIMP-2 protein content ($F = 4.62$, $p = 0.057$). The CIs of the respective ESs did not confirm the difference observed in the relative changes in satellite cell count (ES: 0.82; 95% CI: −0.01 to 1.65) or in TIMP-1 (ES: -0.54; 95% CI: −1.35 to 0.28) but confirmed



LOADprog
REPSprog
(A)
mCSA (%)
*
2×TE = 5.7%
(B)
Volume load (tons)
(C)
Myonuclei per fiber (%)
(D)
Satelite cells per fiber (%)
(E)
Calpain activity (%)
(F)
Calpain-1 protein (%)
(G)
Calpain-2 protein (%)
(H)
p62 protein (%)
(I)
Proteasome activity (%)
(J)
20S Proteasome protein (%)
(K)
Polyubiquitinated proteins (%)
(L)
Global MMP activity (%)
(M)
MMP-9 protein (%)
(N)
MMP-14 protein (%)
(O)
TIMP-1 protein (%)
(P)
TIMP-2 protein (%)

◂**Fig. 2** Relative changes in muscle cross-sectional area and selected biomarkers for "LOADERS". Relative changes observed within the "LOADERS" (n = 7) cluster following 10 weeks of resistance training using LOADProg and REPSProg in **A** muscle cross-sectional area (mCSA); **C** myonuclei and **D** satellite cell count per muscle fiber; enzymatic activity levels of **E** calpain, **I** proteasome and **L** MMPs; and relative protein expression of **F** calpain-1, **G** calpain-2, **H** p62, **J** 20S proteasome, **K** poly-ubiquitinated proteins, **M** MMP-9, **N** MMP-14, **O** TIMP-1 and **P** TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel **B** shows the accumulated volume load, with diamond and circle representing the mean values. LOADERS = cluster of participants whose hypertrophic response to RT met the 2 × TE criterion, and muscle gains favored the LOADProg protocol in over 5.7% (2 × TE) compared to REPSProg; *TE* typical error; *, indicates a significant difference between protocols. Significance was set at $p \leq 0.05$

inferential results for all other outcomes. There was a strong and positive correlation between relative changes in mCSA and satellite cell count per fiber ($r_{rm} = 0.68$; 95% CI: 0.17 to 0.90; $p = 0.016$), and a moderate and negative correlation between relative changes in mCSA and TIMP-1 protein content ($r_{rm} = -0.58$; 95% CI: −0.86 to −0.05; $p = 0.035$). There were no significant correlations with the other assayed markers. The relative changes (post vs. pre), the correlation coefficients and the ES and CI between protocols are presented in the supplemental material (Table 2).

No difference responders

A total of 13 participants (5 women and 8 men) were classified as "NO DIFFERENCE RESPONDERS"; i.e., muscle gains in response to RT met the 2 × TE criterion for at least one of the protocols, but the difference between protocols was smaller than the criterion (5.7%). According to the study design, the ANCOVA results revealed no significant differences when comparing the relative changes in mCSA between REPSProg (14.9 ± 5.6%) and LOADProg (14.5 ± 5.2%; $F = 0.22$, $p = 0.650$) (Fig. 4). The CI of the ES (ES: 0.08; 95% CI: -0.69 to 0.84) confirmed the inferential analysis.

A *t*-test revealed no significant differences between the volume load achieved in LOADProg (53.9 ± 15.3 tons) and that achieved in REPSProg (52.5 ± 14.1 tons; $p = 0.582$) (Fig. 4). The average number of repetitions performed per set per training session was greater for REPSProg (14.4 ± 2.9) than for LOADProg (10.5 ± 0.5; $p < 0.001$), while the average load used per set per session was greater for LOADProg (56.9 ± 14.5 kg) than for REPSProg (40.6 ± 11.0 kg; $p < 0.001$).

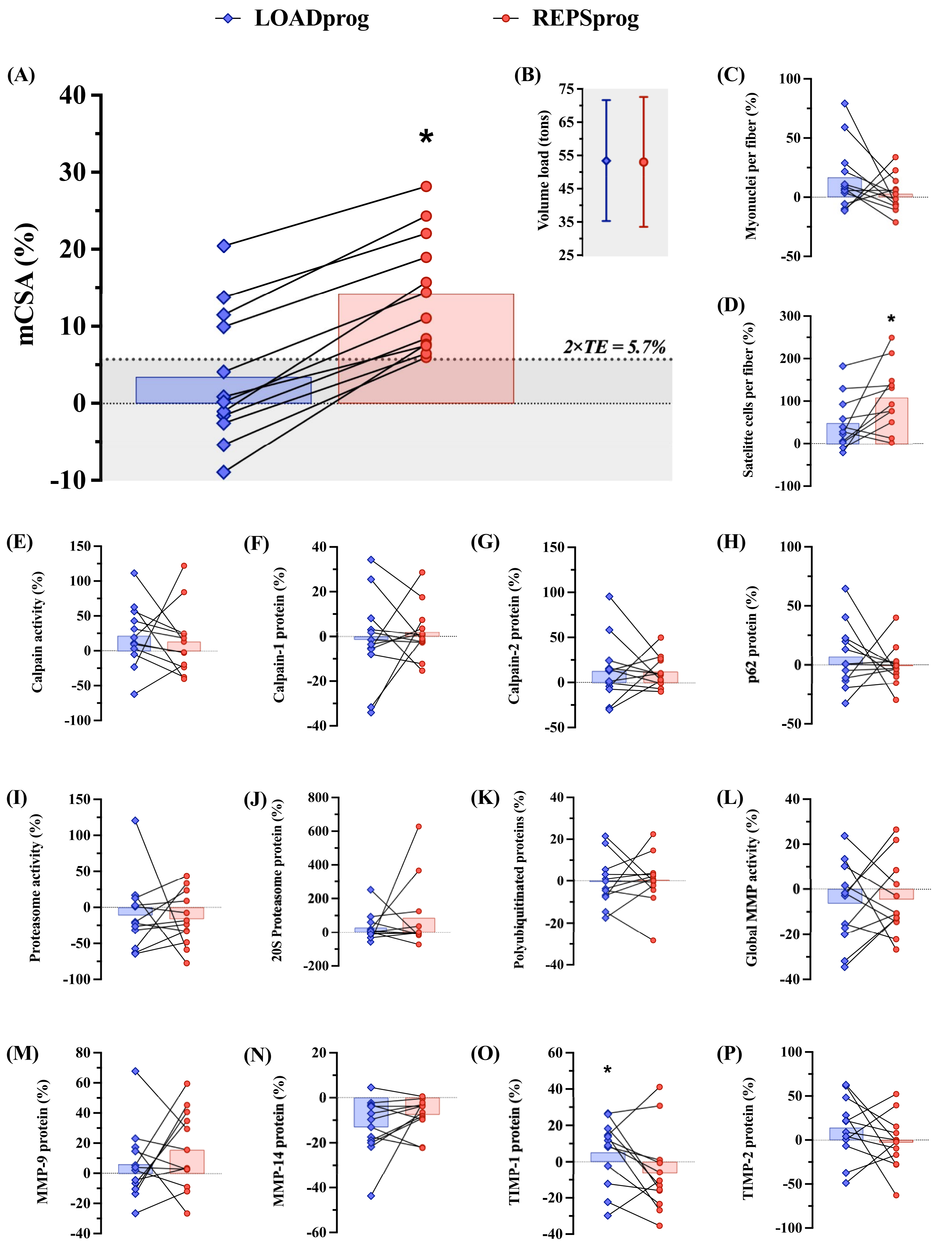
ANCOVA results revealed no significant differences in the assayed markers (Fig. 4): (i) myonuclear number ($F = 0.51$, $p = 0.490$), (ii) satellite cell counts ($F = 2.92$, $p = 0.118$), (iii) global calpain activity ($F = 4.50$, $p = 0.057$), (iv) global proteasome activity ($F = 1.25$, $p = 0.287$), (v) global MMP activity ($F = 0.17$, $p = 0.692$), (vi) calpain-1 protein content ($F = 0.04$, $p = 0.846$), (vii) calpain-2 protein content ($F = 3.78$, $p = 0.078$), (viii) 20S proteasome protein content ($F = 0.01$, $p = 0.919$), (ix) polyubiquitinated proteins ($F = 2.91$, $p = 0.116$), (x) p62 protein content ($F = 1.26$; $p = 0.285$), (xi) MMP-9 protein content ($F = 0.49$, $p = 0.500$), (xii) MMP-14 protein content ($F = 0.54$, $p = 0.480$), (xiii) TIMP-1 protein content ($F = 3.75$, $p = 0.079$), or TIMP-2 protein content ($F = 4.29$, $p = 0.063$). The CI of the respective ES confirmed the absence of difference observed in most of the markers but indicated significant effects in the protein content of TIMP-1 (ES: 1.14; 95% CI: 0.31 to 1.97) and TIMP-2 (ES: 0.81; 95% CI: 0.01 to 1.61), both favoring changes observed in REPSProg rather LOADProg. There were no significant correlations between changes in mCSA and in the assayed markers. The relative changes (post vs. pre), the correlation coefficients and the ES and CI between protocols are presented in the supplemental material (Table 3).

Nonresponders

Five participants (4 women and 1 man) were considered "NONRESPONDERS"; i.e., muscle gains were smaller than 5.7% in response to both protocols. According to the study design, the ANCOVA results revealed no significant differences in the mCSA relative changes between LOADProg (1.1 ± 3.6%) and REPSProg (−2.5 ± 6.6%; $F = 0.92$, $p = 0.408$) (Fig. 5). The CI of the ES (ES: -0.61; 95% CI: -1.87 to 0.66) confirmed the inferential analysis.

There were no significant differences between the volume load observed in LOADProg (43.9 ± 20.7 tons) and REPSProg (42.6 ± 21.9 tons; $p = 0.241$) (Fig. 5). There were no differences in the average number of repetitions performed (LOADProg: 10.5 ± 0.6; REPSProg: 12.3 ± 1.9 kg; $p = 0.058$) or the average load utilized per set per session (LOADProg: 45.0 ± 20.1 kg; REPSProg: 36.4 ± 12.7 kg; $p = 0.069$).

ANCOVA revealed that changes in TIMP-2 protein content were greater for LOADProg (11.6 ± 32.0%) than for REPSProg (−2.4 ± 35.8%; $F = 20.45$, $p = 0.020$) (Fig. 5). There were no differences in the changes of the following biomarkers (Fig. 5): (i) myonuclear number ($F = 2.61$, $p = 0.205$), (ii) satellite cell counts ($F = 3.48$, $p = 0.159$), (iii) global calpain activity ($F = 0.08$, $p = 0.795$), (iv) global proteasome activity ($F = 2.53$, $p = 0.209$), (v) global MMP activity ($F = 2.83$, $p = 0.191$), (vi) calpain-1 protein content ($F = 4.21$, $p = 0.132$), (vii) calpain-2 protein content ($F = 1.46$, $p = 0.314$), (viii) 20S proteasome protein content ($F = 1.01$, $p = 0.389$), (ix) polyubiquitinated proteins ($F = 0.00$, $p = 0.951$), (x) p62 protein content ($F = 2.32$, $p = 0.225$), (xi) MMP-9 protein content ($F = 0.13$, $p = 0.740$), (xii) MMP-14 protein content ($F = 0.05$, $p = 0.830$), or (xiii)



LOADprog
REPSprog
(A)
mCSA (%)
2×TE = 5.7%
*
(B)
Volume load (tons)
(C)
Myonuclei per fiber (%)
(D)
Satellite cells per fiber (%)
*
(E)
Calpain activity (%)
(F)
Calpain-1 protein (%)
(G)
Calpain-2 protein (%)
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(L)
Global MMP activity (%)
(M)
MMP-9 protein (%)
(N)
MMP-14 protein (%)
(O)
TIMP-1 protein (%)
*
(P)
TIMP-2 protein (%)

◂**Fig. 3** Relative changes in muscle cross-sectional area and selected biomarkers for "REPERS". Relative changes observed within the "REPERS" (n = 12) cluster following 10 weeks of resistance training using LOADProg and REPSProg in **A** muscle cross-sectional area (mCSA); **C** myonuclei and **D** satellite cell count (n = 11) per muscle fiber; enzymatic activity levels of **E** calpain, **I** proteasome and **L** MMPs; and relative protein expression of **F** calpain-1, **G** calpain-2, **H** p62, **J** 20S proteasome, **K** poly-ubiquitinated proteins, **M** MMP-9, **N** MMP-14, **O** TIMP-1 and **P** TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel **B** shows the accumulated volume load, with diamond and circle representing the mean values. REPERS = cluster of participants whose hypertrophic response to RT met the 2 × TE criterion, and muscle gains favored the REPSProg protocol in over 5.7% (2 × TE) compared to LOADProg; *TE* typical error; *, indicates a significant difference between protocols. Significance was set at $p \leq 0.05$

TIMP-1 protein content ($F = 2.02$, $p = 0.250$) (Fig. 5). The CIs of the ESs did not confirm the difference observed in the relative changes in TIMP-2 protein content (ES: -0.37; 95% CI: -1.62 to 0.88) but confirmed the remaining results of the inferential analysis. There were no significant correlations between changes in mCSA and in the assayed markers. The relative changes (post vs. pre), the correlation coefficients and the ES and CI between protocols are presented in the supplemental material (Table 4).

Cluster distribution analysis

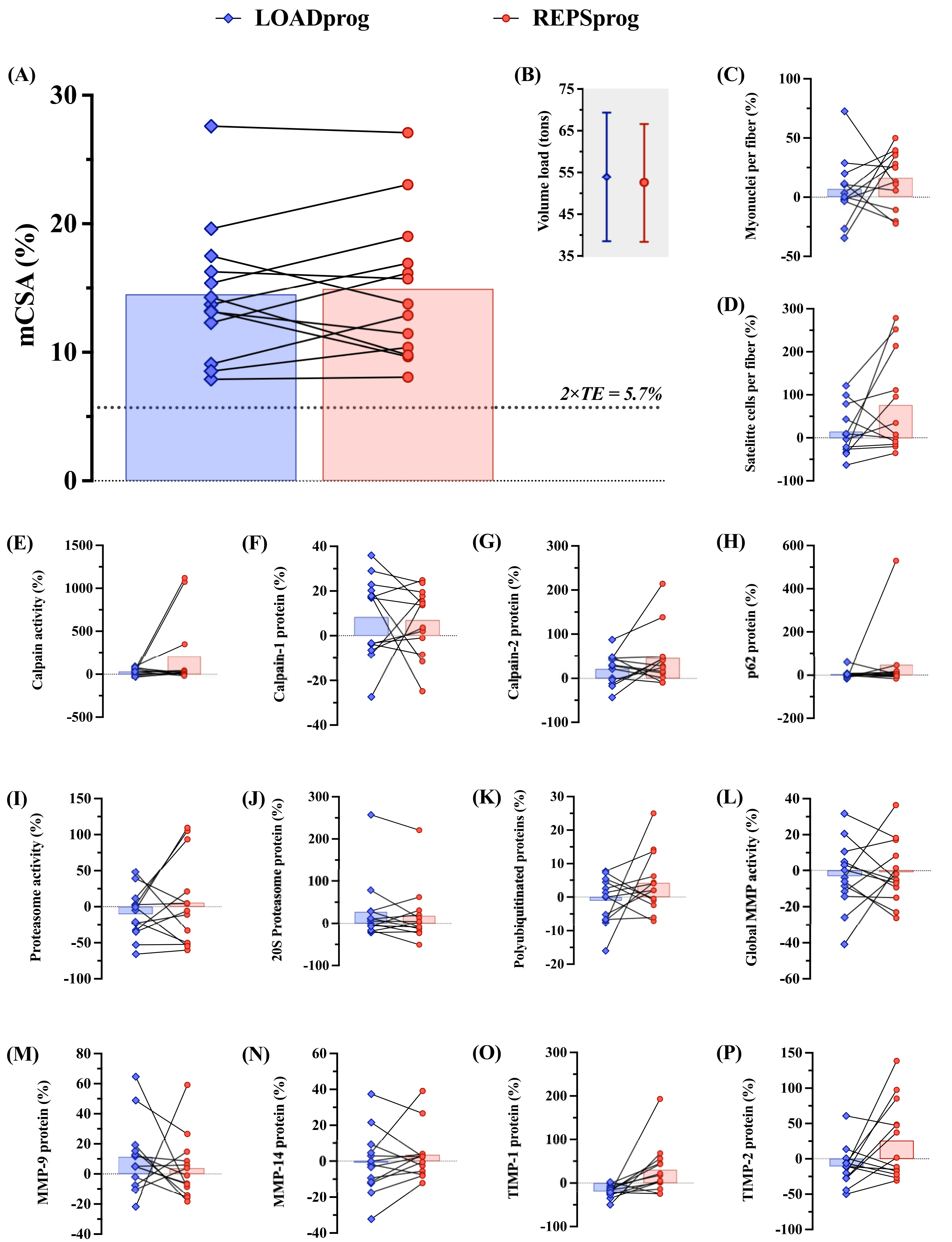
The result of the chi-square test of goodness-of-fit reveals that the distribution of participants between REPERS and LOADERS was not significantly different (χ^2 [3, n = 37] = 1.316, $p = 0.725$). Considering that the chi-square test is sensitive to the sample size (McDonald 2014), we have performed simulations to verify the sample size that would be necessary to observe a significant difference. Simulation analyses indicate that in a sample size greater than 222 participants, the same frequency amongst clusters would result in a significantly different distribution of participants between LOADERS and REPERS (χ^2 [3, n = 222] = 7.895, $p = 0.048$).

Discussion

The current study is the first to investigate individual RT-induced responses to different models of overload progression. We were able to explore the variability in responsiveness due to the use of a within-subject design, with each participant performing both the LOADProg and REPSProg protocols. Notably, one-third of participants presented greater muscle hypertrophy when overload progressed by increasing repetitions compared to increasing load, which was associated with chronic changes in the number of satellite cells.

For a considerable portion of our sample (n = 22 [~ 60%]), both overload progression models were effective at promoting muscle hypertrophy greater than the 2 × TE criterion, which warrants a high probability of true adaptation. Given that muscle mass gain, regardless of its magnitude, contributes to health benefits, both LOADprog and REPSprog appear to be viable approaches. On the other hand, some participants (n = 5 [13.5%]) were resistant to the training stimuli, which has been reported to occur in numerous other studies (Bamman et al. 2007; Petrella et al. 2008; Ahtiainen et al. 2016; Stec et al. 2016; Damas et al. 2019; Lixandrão et al. 2024). While non-responsiveness can be broadly affected by genetic factors and nutritional status (Mann et al. 2014), it is possible that the training stimuli provided by both overload progression models used in the present study were insufficient to activate muscle hypertrophy-related molecular mechanisms (Roberts et al. 2023). Although it is unknown whether these individuals would benefit from different RT stimuli, Lixandrão et al. (2024) recently reported that increased volume mitigated non-responsiveness in older adults. Hence, it is possible that other RT protocols could trigger muscle growth in nonresponders.

When comparing muscle hypertrophy between protocols, our results indicate that 35.1% of the participants showed no differences between them ("NO DIFFERENCE RESPONDERS"). Those findings are consistent with our previous group analysis which showed no differences in muscle hypertrophy following LOADProg and REPSProg (Chaves et al. 2024). Nonetheless, most of our participants (51.3%) showed muscle gains favoring one or the other protocol, with responses differing by more than 5.7% (2 × TE). It is striking that REPSProg promoted greater mCSA increases to 12 (~ 32.4%) participants ("REPERS"), while the conventional overload progression model (i.e., LOADProg) favored just approximately half of that amount (n = 7 [~ 18.9%]; "LOADERS"). Furthermore, for some individuals, the overload progression protocol was a key determinant for reaching the responsivity threshold since 8 of the 12 "REPERS" responded only to REPSProg (i.e., changes in the mCSA of the leg that performed LOADProg within the non-responder threshold), and 2 of the 7 "LOADERS" responded exclusively to LOADProg. These differences were observed despite the similar volume loads achieved. However, the design of the protocols led to differences in the volume load components, with a greater volume (number of repetitions) being performed during REPSProg and greater loads being performed in LOADProg. Although the chi-squared test did not indicate a statistically significant difference in the number of participants classified as REPERS and LOADERS, it is important to note that this test is sensitive to the sample size (McDonald 2014). In a larger sample (n ≥ 222), the same distribution of participants among clusters would likely result in a statistically



LOADprog
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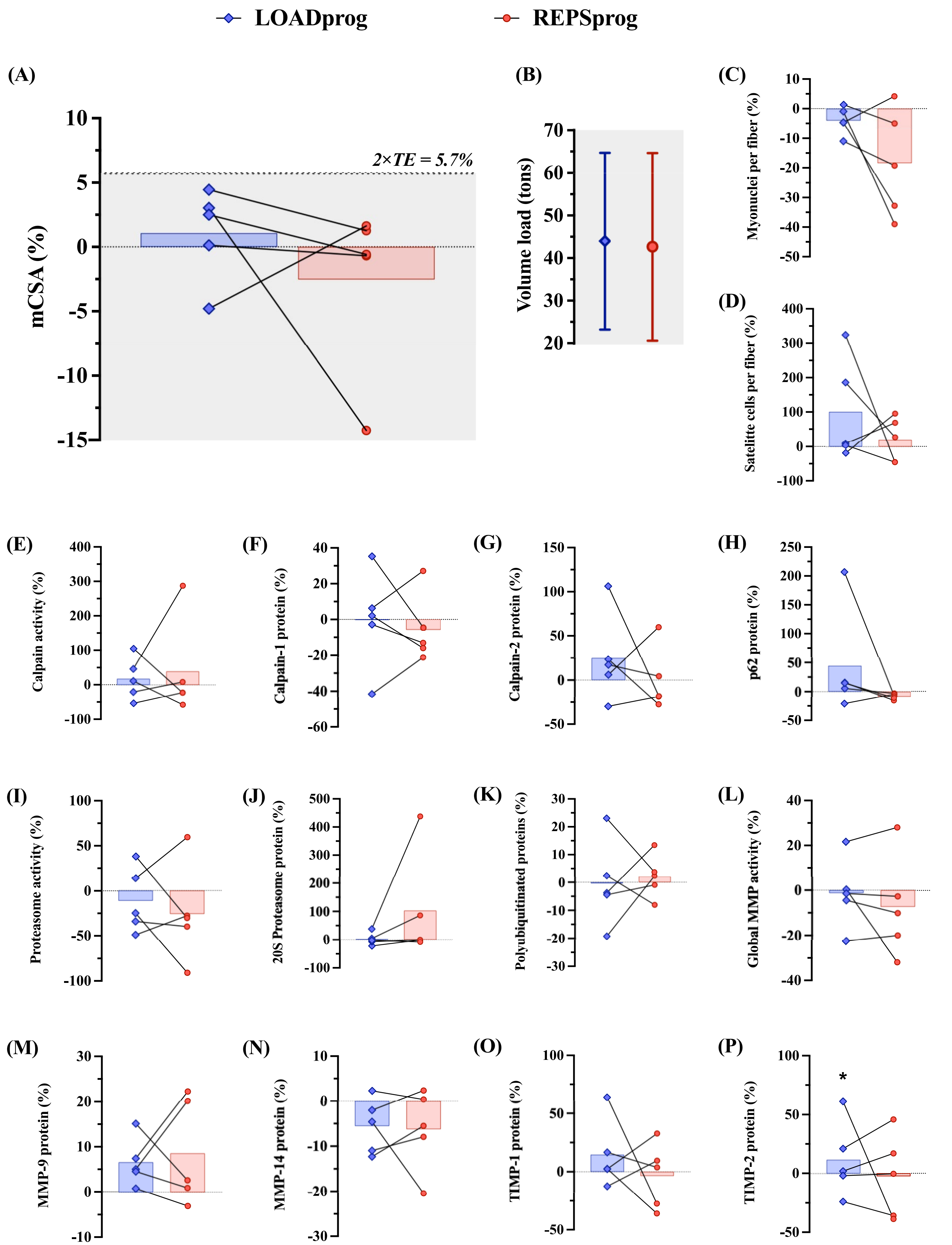
◂**Fig. 4** Relative changes in muscle cross-sectional area and selected biomarkers for "NO DIFFERENCE RESPONDERS". Relative changes observed within the "NO DIFFERENCE RESPONDERS" (n = 13) cluster following 10 weeks of resistance training using LOADProg and REPSProg in **A** muscle cross-sectional area (mCSA); **C** myonuclei (n = 12) and **D** satellite cell count (n = 12); enzymatic activity levels of **E** calpain, **I** proteasome and **L** MMPs; and relative protein expression of **F** calpain-1, **G** calpain-2, **H** p62, **J** 20S proteasome, **K** poly-ubiquitinated proteins, **M** MMP-9, **N** MMP-14, **O** TIMP-1 and **P** TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel **B** shows the accumulated volume load, with diamond and circle representing the mean values. NO DIFFERENCE RESPONDERS = cluster of participants whose hypertrophic response to RT met the 2 × TE criterion but the difference between protocols was smaller than 5.7% (2 × TE); *TE* typical error; *, indicates a significant difference between protocols. The significance level was set at $p \leq 0.05$

significant difference between the number of LOADERS and REPERS. While this suggests that REPSProg may have the potential to elicit comparable or even greater muscle hypertrophy in a larger proportion of individuals, it is important to acknowledge that the present study did not find a statistically significant difference in responder distribution. Therefore, further research with larger samples is required to determine whether higher volumes (i.e., more repetitions) provide a hypertrophic advantage over higher loads in a broader population. Our study is the first to explore, via a within-subject approach, whether biomarkers of molecular processes are differentially modulated by RT progression protocols and are associated with divergent hypertrophic responses. Studies that have compared individual responses to RT protocols have not investigated molecular markers (Damas et al. 2019; Angleri et al. 2022b; Lixandrão et al. 2024). Even so, individual responses and molecular mechanisms have been investigated in low and high responders to the same RT protocol (Bamman et al. 2007; Petrella et al. 2008; Davidsen et al. 2011; Ogasawara et al. 2016; Stec et al. 2016; Mobley et al. 2018; Haun et al. 2019; Godwin et al. 2023; Smith et al. 2023). For example, myonuclear accretion mediated by satellite cells is a primary mechanism associated with the individual variability in hypertrophic responses (Petrella et al. 2006, 2008; Roberts et al. 2018; Angleri et al. 2022a). Petrella et al. (2008) classified young and older participants of both sexes without previous RT experience into three clusters based on myofiber hypertrophy. After 16 weeks of RT, extreme responders expanded their myonuclei and satellite cell pool, while nonresponders did not. Similarly, in a cohort of 19 young resistant trained men, Angleri et al. (2022a) reported that changes in muscle fiber cross-sectional area were aligned with changes in the number of myonuclei and in the satellite cell pool in the higher responders and lower responders. Our findings corroborate the results for satellite cells but not for myonuclei. The greater hypertrophy observed for REPSProg in the REPERS cluster was accompanied by a greater addition of satellite cells compared to LOADProg (Fig. 3, Panel D), and there was a positive correlation between these variables ($r_{rm} = 0.68$). In contrast, the small number of participants may have hindered this observation in LOADERS. Regarding the changes in myonuclei count, Petrella et al. (2008) and Angleri et al. (2022a) compared clusters of high and low responders with critically contrasting hypertrophic responses. Myofiber hypertrophy averaged 58 and 0% respectively for extreme responders and nonresponders in Petrella et al. (2008), and 39 and -9% respectively for the top 4 responders and bottom 4 lower responders in Angleri et al. (2022a). Meanwhile, our analyses compared RT protocols that promoted muscle hypertrophy, which differed by an average of ~ 10% between protocols (for "REPERS" and "LOADERS"). In fact, a more robust muscle hypertrophy seems to be associated with greater myonuclear accretion (Conceição et al. 2018). Although our study lasted 10 weeks, which is comparable to other investigations on RT adaptations, studies reporting significant increases in myonuclear content often employed longer interventions (e.g., ≥ 16 weeks, Petrella et al. 2008). It is possible that a more prolonged training period could have promoted greater muscle hypertrophy, thereby enhancing myonuclear accretion and potentially revealing an association between these variables. Thus, we hypothesize that any differences in myonuclei count that could arise due to a participant-by-training interaction would depend on observing a greater contrast in muscle adaptations, especially in response clusters of small size.

Both proteolytic activity and ECM remodeling seem to be involved in the regeneration of skeletal muscle (Pasiakos and Carbone 2014; Ahmad et al. 2023; Roberts et al. 2023). Moreover, proteolysis may be critical for muscle growth, as the inhibition or downregulation of calpain or the proteasome seem to impair muscle or myotube hypertrophy (Baehr et al. 2014; Kitajima et al. 2014; Osburn et al. 2021). Although proteolysis has been speculated to be a possible factor driving differential individual responses, the evidence is sparse (Roberts et al. 2018). Some studies have made inferences about proteolytic activity derived from inflammation markers, considering that chronic elevations in skeletal muscle pro-inflammatory cytokines (e.g., IL-6, TNF-α, and IL-1β) seem to upregulate muscle proteolysis (Zamir et al. 1992; Haddad et al. 2005; Raj et al. 2008). From an individual response perspective, one's inability to control inflammatory signaling post-exercise could lead to heightened muscle proteolysis, which by its turn would hinder muscle growth (Roberts et al. 2018). Nevertheless, the evidence regarding the association between inflammation and individual responsiveness to RT is equivocal. For instance, Mobley et al. (2018) reported that while IL-1β mRNA was downregulated in high responders compared with low responders, other inflammatory markers (e.g., IL-6 and TNF-α mRNA) were not differentially expressed. The same study showed



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*

◂**Fig. 5** Relative changes in muscle cross-sectional area and selected biomarkers for “NONRESPONDERS”. Relative changes observed within the “NONRESPONDERS” (n = 5) cluster following 10 weeks of resistance training using LOADProg and REPSProg in **A** muscle cross-sectional area (mCSA); **C** myonuclei and **D** satellite cell count per muscle fiber; enzymatic activity levels of **E** calpain, **I** proteasome and **L** MMPs; and relative protein expression of **F** calpain-1, **G** calpain-2, **H** p62, **J** 20S proteasome, **K** poly-ubiquitinated proteins, **M** MMP-9, **N** MMP-14, **O** TIMP-1 and **P** TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel **B** shows the accumulated volume load, with diamond and circle representing the mean values. NONRESPONDERS = cluster of participants whose muscle gains were smaller than 5.7% (2 × TE) in response to both protocols; *TE* typical error; *, indicates a significant difference between protocols. Significance was set at p ≤ 0.05

that a cluster × time interaction approached significance for 20S proteasome activity ($p = 0.058$), indicating that low hypertrophy responders could have heightened proteolytic activity. However, the authors acknowledge that the plausibility of this hypothesis is limited by the lack of association between changes in 20S proteasome levels and changes in hypertrophy. To date, no study has investigated proteolysis markers from an individual response perspective comparing RT protocols. Notably, we assessed markers of protein content and enzymatic activity related to three different proteolysis pathways (i.e., autophagy, the calpain pathway and the ubiquitin−proteasome pathway). Our findings revealed that none of the associated markers were differentially modulated between the RT overload progression models. Thus, our results support a lack of association between RT responsiveness and certain markers of proteolytic activity.

There is also a paucity of studies exploring individual RT-responsiveness and markers associated with the remodeling of muscle ECM. Our findings suggest that the protein content of TIMP-1 may be differentially modulated between overload progression models, indicating that ECM turnover may respond differently depending on the applied training stimulus. TIMP-1 is a key regulator of ECM homeostasis, acting as an inhibitor of matrix metalloproteinases (MMPs) and contributing to the balance between ECM degradation and synthesis (Roberts et al. 2023). While the functional implications of this finding remain unclear, this observation underscores the need for further studies to clarify the role of ECM remodeling in hypertrophic adaptations. Yet, all other ECM remodeling markers were not differentially modulated, corroborating results from previous studies (Angleri et al. 2022a; Godwin et al. 2023). Godwin et al. (2023), using a between-subject design, showed no differential training responses in most skeletal muscle ECM markers when comparing low and high responders to 10 weeks of RT. Similarly, Angleri et al. (2022a) revealed no association between individual increases in total fCSA and gene expression of ECM remodeling markers. In addition, the study presents a heatmap stratified by myofiber hypertrophy that allows a qualitative analysis of the individual responses to two different RT protocols (Angleri et al. 2022a). It is clear that the variance observed in myofiber hypertrophy is not reflected in the gene expression of ECM remodeling markers in response to those protocols. Given that individual hypertrophic responses are influenced by a multifactorial interplay of molecular processes (Roberts et al. 2018), further research is needed to determine whether ECM remodeling is involved in modulating responsiveness to different RT progression strategies. Future studies should explore the temporal dynamics of ECM regulation and its potential interaction with other hypertrophy-related mechanisms.

Like others, this study is not without limitations. Our sample comprised exclusively young healthy untrained participants, thus precluding the extrapolation of the results to other populations. Additionally, RT protocols were performed over a limited 10-week period. It is uncertain whether responsiveness favoring one or the other protocol would be altered if RT practice continued for a longer period. Although this duration is comparable to previous studies on RT-induced adaptations, those reporting significant myonuclear accretion have often employed longer interventions. Thus, an extended training period might have further enhanced muscle hypertrophy, potentially leading to greater myonuclear accretion and a clearer association between these variables. Furthermore, our analysis of hypertrophic responses was based on mCSA changes, as applying the 2 × TE criterion to muscle fiber cross-sectional area would have required repeated pre-training biopsies, which were not feasible within our study design. This limitation should be considered when interpreting our findings, as fiber-type-specific hypertrophy may exhibit distinct variability patterns. Finally, it is worth noting that this dichotomous classification clustering procedure resulted in groups of relatively small sizes and low power for detecting significant effects. While this may limit statistical power for detecting smaller effect sizes, the within-subject design helped mitigate interindividual variability, improving the sensitivity of our analysis. Future studies with larger samples will be valuable in confirming these findings and further investigating the mechanisms underlying individual responsiveness to RT progression models.

Conclusion

Our results suggest that the overload progression models may affect responsiveness to muscle hypertrophy induced by RT. Notably, it is intriguing that, the progressing overload by increasing the number of repetitions resulted in associated with greater chronic addition of satellite cells. However, overload progression models did not differentially affect chronic changes in myonuclei, proteolysis activity markers, or markers associated with ECM remodeling. Future studies should investigate other molecular mechanisms that may explain the variability in individual responsiveness to different models of RT progression, especially to understand the hypertrophic response resulting from the progression of overload due to the increase in the number of repetitions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00421-025-05817-y.

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Author contributions M.C.S. and C.A.L. conceived and designed the idea for this ancillary analysis. M.C.S., J.G.B., and T.S.C. recruited participants and coordinated training sessions and data collection. D.G.S., D.F.B., J.G.B., N.F.D., P.C.C.F., R.M.J., T.S.C., V.A. and L.A.R.C. performed training sessions and data collection. M.C.S., J.G.B., J.S.G. and P.H.C.M. conducted laboratory assays and experiments. M.C.S., C.A.L. and M.D.R. analyzed and interpreted the data. M.C.S. primarily drafted the manuscript, and C.A.L. and M.D.R. edited it. All authors revised the manuscript and provided intellectual contributions. All authors approved the final version of the manuscript. All authors qualify for authorship, and all those who qualify are listed.

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Data availability Raw data will be made available by the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest related to the content of this article.

Ethical approval The study was approved by the local ethics committee and conducted in accordance with the most recent version of the Declaration of Helsinki.

Consent to participate Informed consent was obtained from all individual participants included in the study.

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