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Resistance training variable manipulations are less relevant than intrinsic biology in affecting muscle fiber hypertrophy

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We aimed to investigate whether muscle fiber cross-sectional area (fCSA) and associated molecular processes could be differently affected at the group and individual level by manipulating resistance training (RT) variables. Twenty resistance-trained subjects had each leg randomly allocated to either a standard RT (RT-CON: without specific variables manipulations) or a variable RT (RT-VAR: manipulation of load, volume, muscle action, and rest interval at each RT session). Muscle fCSA, satellite cell (SC) pool, myonuclei content, and gene expression were assessed before and after training (chronic effect). Gene expression was assessed 24 h after the last training session (acute effect). RT-CON and RT-VAR increased fCSA and myonuclei domain in type I and II fibers after training ($p < 0.05$). SC and myonuclei content did not change for both conditions ($p > 0.05$). Pax-7, MyoD, MMP-2 and COL3A1 (chronic) and MGF, Pax-7, and MMP-9 (acute) increased similar for RT-CON and RT-VAR ($p < 0.05$). The increase in acute MyoG expression was significantly higher for the RT-VAR than RT-CON ($p < 0.05$). We found significant correlation between RT-CON and RT-VAR for the fCSA changes ($r = 0.89$). fCSA changes were also correlated to satellite cells ($r = 0.42$) and myonuclei ($r = 0.50$) changes. Heatmap analyses showed coupled changes in fCSA, SC, and myonuclei responses at the individual level, regardless of the RT protocol. The high between and low within-subject variability regardless of RT protocol suggests that the intrinsic biological factors seem to be more important to explain the magnitude of fCSA gains in resistance-trained subjects.

KEYWORDS
biological predisposition, individual responses, resistance exercise, responsiveness, satellite cells, training variables

1 | INTRODUCTION

Skeletal muscle mass plays a vital role in health, sports performance, and esthetics.[1,2] Resistance training (RT) is the most effective method to increase muscle fiber cross-section area (fCSA) (i.e., muscle hypertrophy).[3–5] Frequent manipulation of RT variables (e.g., load, volume, muscle action, and rest) is widely suggested to differently potentiate fiber type-specific CSA increases compared to standard progressive RT, especially for resistance-trained individuals.[5–10] Theoretically, manipulating RT variables would potentiate the response of intrinsic biological factors (e.g., increase in satellite cell pool, myonuclei number, and gene expression) controlling skeletal muscle hypertrophic response.[1,11–15] Nevertheless, it was recently demonstrated similar vastus lateralis muscle hypertrophy between a RT protocol with frequent manipulation of several training variables as compared with a standard linear progressive RT one, at last, when both protocols are performed to (or close to) concentric muscle failure.[16] However, there is a paucity of data on the effect of frequent manipulation of RT variables at the fCSA level and the previously mentioned intrinsic biological factors in resistance-trained individuals.

A large between-subject variability has been observed in the RT-induced increase in fCSA, when comparing distinct RT protocols.[12-14,17,18] Accordingly, we recently demonstrated higher between- than within-subject variability in myofibrillar protein synthesis and muscle hypertrophy, regardless of the RT protocol, using a within-subject design (i.e., one training protocol for each leg).[16] These findings suggest that intrinsic biological factors, rather than extrinsic ones (e.g., manipulating RT variables), are mainly mediating the magnitude of muscle hypertrophic response between high and low responders. Among the hypertrophy-related intrinsic factors, the SC has received particular attention,[12–14,18] as they donate myonuclei to muscle fibers to support the increased protein synthesis demand of an expanding cytoplasmic volume.[12,14,19] This process is coordinated by an increased expression of genes related to the SC cycle and its niche (e.g., matrix metalloproteinases [MMP-2 and MMP-9], mechanogrowth factor [MGF], paired box protein 7 [Pax-7], myoblast determination protein 1 [MyoD], and myogenin [MyoG]) induced by both a single and several RT sessions (acute and chronic effect).[12,13,20-22] Importantly, it was demonstrated that the magnitude of the change in the expression of some of the aforementioned genes[13] and SC-mediated myonuclei addition[14] were aligned with the magnitude of increase in fCSA induced by a standard RT program, in individuals with no previous RT experience. Although these findings suggest that between-subject variability can explain the differences in adaptive response, they do not determine if the frequent variation of RT-related variables can decrease the variability in the change of these intrinsic biological factors between high and low responders.

This study aimed to investigate whether intrinsic biological factors (i.e., SC response, myonuclei addition, and gene expression) could be modulated by frequent manipulations of RT variables and potentiate the gains in fCSA in resistance-trained young men. We also investigated whether within-subject variability in fCSA and intrinsic biological factors could be modulated by RT variables manipulations and whether these intrinsic factors could be related to the magnitude of increase in fCSA. To address these aims, we used an unilateral RT, in which one leg performed a standard progressive RT (RT-CON), and the contralateral leg performed a variable RT (RT-VAR) modulating key-exercise variables, such as load, volume, muscle action, and interset rest interval. We hypothesized that: (1) RT-CON and RT-VAR would similarly increase type I and II muscle fCSA; (2) RT-CON and RT-VAR would induce similar SC response, myonuclei addition, and expression of the genes involved in this mechanism; (3) RT protocols would produce a low variability at the within-subject level and large variability in the fCSA increases and the intrinsic biological factors irrespective of RT regimen at the between-subject level; and (4) the intrinsic biological factors would better explain the individual magnitude of muscle fiber hypertrophy, regardless RT modulations.

2 | METHODS

2.1 | Participants

Healthy young resistance-trained men ($n = 20$; age: 26 ± 3 year, body mass index: 25.6 ± 2.1 kg/m^2 and RT experience: 2.5 ± 1.1 year) volunteered to participate in this study. Subjects were considered as trained if they trained for at least 1 year uninterruptedly.[5,16,23,24] Participants had to be free from musculoskeletal disorders and stated they had not taken anabolic steroids in the previous year. All participants signed an informed consent form. The study was conducted according to the Declaration of Helsinki, and the Human Research Ethics Committee of the local university approved the study (#2.226.596).

2.2 | Experimental design

We used a within-subject design in which chronic and acute data were obtained (Figure 1). Briefly, each participant's legs were balanced and randomly assigned to (1) standard progressive control RT (RT-CON, 10 dominant and 10 non-dominant legs) protocol or (2) variable RT (RT-VAR, 10 dominant and 10 non-dominant legs) protocol

that manipulated the RT variables (full descriptions of RT protocols below) on a per-session basis. Participants refrained from exercising for 72 h before the first experimental session. A vastus lateralis muscle biopsy was performed in one of the legs in a randomized fashion immediately before the first RT session (Pre). Subjects performed two weekly RT sessions over 8 weeks (total of 16 RT sessions), in which both protocols were performed. Bilateral vastus lateralis biopsies were performed 96 h after the last training session (Post). Then, subjects performed an additional RT session (i.e., 17th session), and vastus lateralis muscle samples were harvested from each leg 24 h after it. Biopsies Pre and Post were used to assess chronic changes in fCSA, SC, myonuclei, and gene expression. Biopsies Post (for these analyses now named as 0 h) and 24 h determined the acute effect of the 17th training session. In the acute RT session, participants performed the RT-CON protocol with the same leg they used in the RT-CON program ($n = 20$), and the contralateral leg was randomly assigned to one of the four RT-VAR conditions ($n = 20$; $n = 5$ per condition). Although subjects had previous RT experience, the acute trial was performed only at the end of training protocol to mitigate any bias produced by an unaccustomed exercise stimulus. Participants consumed 30 g of isolated whey protein provided by the researcher after each RT session, including the acute trial (i.e., 17th RT session).

2.3 | Muscle biopsy

Vastus Lateralis muscle biopsies were performed using percutaneous biopsy needles with suction under local anesthesia (2–3 ml of 1% Xylocaine). A portion of 20–30 mg was placed in optimum cutting temperature (OCT) with fibers perpendicular to the horizontal surface and frozen with isopentane cooled by liquid nitrogen to perform fCSA, fiber distribution, SC, and myonuclear content and myonuclear domain per fiber type analyses. Approximately 50 mg of muscle tissue was separated for gene expression analyses. All samples were stored at −80°C until analysis.

2.4 | Resistance training

Both RT protocols were performed to (or near to) concentric muscle failure. The RT-CON performed 8 sets (4 sets of leg press followed by 4 sets of leg extension) with 9–12 maximum repetitions, dynamic muscle actions (concentric and eccentric), and 2-min rest interval between sets and exercises. The load was increased or decreased to maintain the repetition range as previously described.[16] For RT-VAR, one of the following RT manipulations was performed: (1) load: 4 sets of both leg press and leg extension of 25-30RM with 2-min rest interval; (2) volume: 6 sets of leg press and 6 sets of leg extension of 9-12RM with 2-min rest interval; (3) muscle action: 4 sets of leg press and 4 of leg extension of 10 eccentric contractions at 110% of the load used in the RT-CON with 2-min rest interval; and (4) rest: 4 sets of leg press and 4 of leg extension with 9-12RM with 4-min rest interval. As 16 RT sessions were performed during the 8 weeks, this sequence of RT-VAR protocols was repeated 4 times by each participant in a randomized and balanced order.

2.5 | Immunohistochemical analyses

Frozen muscle samples were sectioned using a cryostat (Leica CM 1860) at −25°C. The slides with muscle cross-sections (6 μm) were left at room temperature for ~20 min

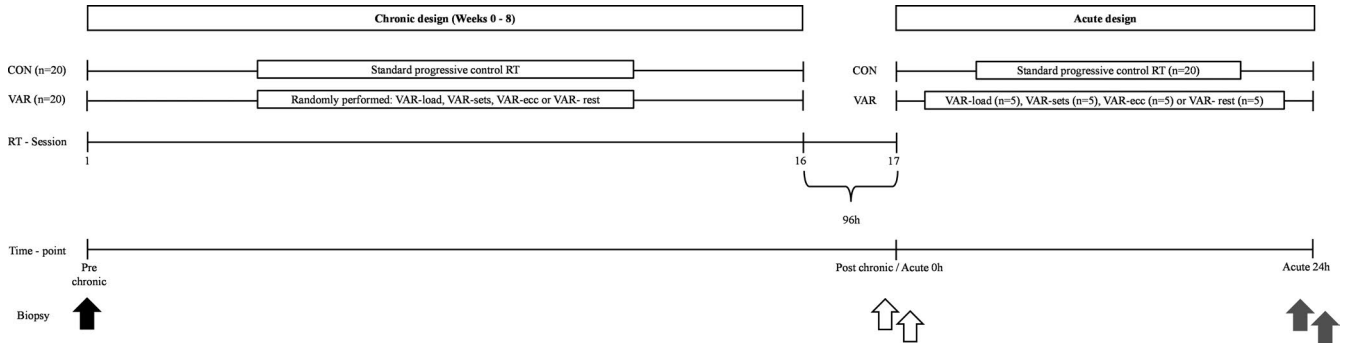


FIGURE 1 Experimental design. Double arrows indicate bilateral muscle biopsies. The black arrow indicates biopsy utilized for the Pre measurements of the chronic design; white arrows indicate biopsies utilized for the Post measurements in the chronic design (96 h after the 16th RT session) and 0 h (immediately before the 17th RT session) measurements in the acute design; and gray arrows indicate biopsies utilized for the 24 h post 17th RT session analyses in the acute design. RT: resistance training; CON: control RT (4 sets for both leg press and leg extension, 9-12RM/2-min rest); VAR: variable RT; VAR-load: 4 sets for both leg press and leg extension, 25-30RM/2-min rest; VAR-sets: 6 sets for both leg press and leg extension, 9-12RM/2-min rest; VAR-ecc: 4 sets for both leg press and leg extension, 10 eccentric contractions at 110% of the load used in CON leg/2-min rest; VAR-rest: 4 sets for both leg press and leg extension, 9-12RM/4-min rest

to stabilize. Samples were incubated with the primary antibodies at 37°C for 45 min. The samples were washed 3 times for 5 min in phosphate-buffered saline (PBS) and then were incubated with the secondary antibodies at 37°C for 45 min. After 3 more 5 min washes in PBS, the sections were mounted in FluorQuest with DAPI. The images were obtained with an ImageXpress Micro XLS with a magnification of 20x. The analyses were performed in the ImageJ software. Due to a problem in the image acquisition for one sample, the subsequent procedures were conducted with 19 subjects. The fCSA was determined using computerized planimetry for each fiber type. To ensure that only cross-sectioned fibers were analyzed, fibers with circularity below 0.60 were excluded from the analysis.[11,25] For type I fCSA, the number of fibers analyzed was 48 ± 6 at Pre, 44 ± 12 at Post (RT-CON), and 47 ± 7 at Post (RT-VAR). Regarding type II fCSA, the number of fibers analyzed was 50 ± 0 at Pre, 47 ± 9 at Post (RT-CON), and 50 ± 1 at Post (RT-VAR). The typical error (TE) between two measurements performed 72 h apart was 116.8 μm^2; 219.0 μm^2; and 175.9 μm^2 for fCSA from type I and II muscle fibers, and total fCSA (fCSA I + fCSA II), respectively. The number of myonuclei for each fiber type was counted and normalized by the number of analyzed fibers. The TE between two measurements performed 72 h apart was 0.15 myonuclei/fiber, 0.13 myonuclei/fiber, and 0.17 myonuclei/fiber for fiber type I, type II, and total (type I + type II) myonuclei number, respectively. The ratio between fCSA and the normalized number of myonuclei was determined as myonuclear domain. The number of SC of specific fiber types was determined by co-localization of DAPI and Pax-7. The fiber type-specific SC was normalized per number of specific fiber type. The TE between two measurements performed 72 h apart was 0.02 SC/fiber for SC of fiber type I, type II, and total (type I + II). The number of type I fibers analyzed for SC and myonuclei count were 97 ± 49 at Pre, 95 ± 55 at Post (RT-CON), and 128 ± 88 at Post (RT-VAR). Finally, the number of type II fibers analyzed for SC and myonuclei count were 144 ± 85 at Pre, 136 ± 86 at Post (RT-CON), and 160 ± 97 at Post (RT-VAR).

2.6 | qRT-PCR

The RNA was isolated, incubating, and homogenizing ~15 mg of muscle tissue in the Trizol reagent (Invitrogen Corporation, California, USA) according to the manufacturer's instructions. RNA concentration and purity and concentration were determined by assessing the absorbance (NanoDrop 2000) of each sample at 260 and 260/280 nm, respectively. The RNA integrity of all samples was certificated by electrophoresis on 1% agarose gel with a pattern of 28S and 18S ribosomal RNA. The samples were treated with DNase I (Invitrogen Corporation California, USA) to remove contaminants of genomic DNA. The treated RNA (1 μg) was reverse transcribed using GoScript™ Reverse Transcriptase (Promega Corporation, Madison, WI, USA). The real-time PCR (CFX 96 real-time PCR–Bio-Rad, San Francisco, USA) was performed using 20 ng of cDNA, and 0.5 μM of each primer was used in 25 μl volume system mix containing SoFastTM Eva Green (BioRad, San Francisco, USA). Samples were analyzed in duplicate. Thermal cycling was 95°C for 10 min, 40 cycles of 95°C for 15 s, 54.4–63.3°C for 30 s, and 72°C for 30 s, respectively. To ensure that only one PCR product was amplified per reaction, the melting curve was completed after PCR. Gene expression fold changes were calculated using the delta–delta Ct method.[26] We tested 4 genes (RPLP0, RPL13A, TFRC, and GAPDH) as housekeeping and used the GAPDH to normalize Ct values (delta-Cts [ΔCt]). Then, the values were used to normalize the ΔCt values (delta-delta Cts [ΔΔCt]). The values were transformed out of the logarithmic scale the equation: fold change $= 2^{-\Delta\Delta Ct}$.[26]

2.7 | Statistical analysis

Chronic effects of RT-CON and RT-VAR on fCSA, SC, and myonuclei content and gene expression were analyzed through two procedures. As Pre data analyses were performed based on the muscle tissue harvested from just one leg, we used a one-way ANOVA to determine whether the dependent variables (fCSA, SC and myonuclei content and chronic gene expression) at Post (RT-CON and RT-VAR) were different from Pre. A Dunnett post hoc test was performed, in case of significant *F*-values, having Pre as the control condition. The same dependent variables were compared between RT-CON and RT-VAR at Post using paired t-tests. Acute changes in gene expression were analyzed using several mixed models assuming time (0 and 24 h) and protocol (RT-CON and RT-VAR) as fixed factors and subjects as a random factor. Pearson's correlation estimated the association of the changes in fCSA, SC and myonuclei between protocols and between them. We also assessed the association of the changes in fCSA with chronic and acute gene expression changes between protocols and legs. As the changes in fCSA ($r = 0.89$) were highly correlated between RT-CON and RT-VAR legs, we collapsed all the data to perform the subsequent correlations ($n = 38$). Thus, we performed Pearson's correlations between total fCSA changes and (i) total satellite cells change; (ii) total myonuclei changes; (iii) chronic gene expression; and (iv) acute gene expression. Heatmaps were used to explore the alignment between muscle fiber hypertrophy and intrinsic biological factors. To generate the heatmaps, the total fCSA (CSA of fibers type I + CSA of fibers type II) of each subject was ranked according to the mean change (Δ%) values

of both legs ([RT-CON +RT-VAR] ÷ 2). Then, the Δ% of the other dependent variables (i.e., total SC [number of SC per type I + II fibers], total myonuclei number [number of myonuclei per type I + type II], chronic [Pre to Post], and acute changes [0 to 24 h] gene expression) per subject were ranked according to the Δ% of total fCSA. The delta change for each heatmap column was normalized individually. For total fCSA, SC and myonuclei heatmaps baseline values were set to 0. The heatmap for the chronic gene expression had the baseline value set as the mean value of Pre gene expression, whereas the acute gene expression had the baseline value set as the mean of RT-CON and RT-VAR gene expression at 0 h. Changes above and below the baseline values were filled with red and blue color, respectively, intense shades of red indicating higher Δ% and more intense shades of blue indicating a decrease in the dependent variable. White shades indicate no changes. Finally, three heatmaps were performed comparing: 1—total fCSA Δ%, total SC Δ%, and total myonuclei content Δ%; 2—total fCSA Δ% and chronic gene expression Δ%; and 3—total fCSA Δ% and acute gene expression Δ%. These analyses allow us to visually explore whether the higher or lower responders for total fCSA were aligned with higher and lower changes on the assessed biological factors and if the RT regimen could modulate this response.

3 | RESULTS

3.1 | Volume load

We found a higher accumulated volume load (sets × reps × load [kg]) for RT-VAR (217,613 ± 43,834 kg) compared with RT-CON (193,259 ± 39,731 kg) (12.6% difference between legs; $p < 0.001$; results not shown in figure).

3.2 | Fiber type distribution, cross-sectional area, satellite cells content, myonuclear content and domain

There were significant increases in type I fCSA for RT-CON (13.0% [$p = 0.01$]) and RT-VAR (12.3% [$p = 0.02$]) (Table 1). Type II fCSA also significantly increased for RT-CON (12.7% [$p = 0.02$]) and RT-VAR (12.2% [$p = 0.03$]) (Table 1). Myonuclear domain size for type I muscle fibers significantly increased for RT-CON (15.4% [$p = 0.0001$]) and RT-VAR (19.9% [$p = 0.0013$]) (Table 1). There were significant increases in myonuclear domain size for type II muscle fiber for RT-CON (23.1% [$p = 0.0002$]) and RT-VAR (24.2% [$p = 0.004$]) (Table 1). No significant changes in fiber type distribution, myonuclear and SC content were found for RT-CON and RT-VAR for both fiber types ($p > 0.05$) (Table 1). Importantly, no significant differences were found between RT-CON and RT-VAR for any dependent variables at post ($p > 0.05$) (Table 1).

3.3 | mRNA gene expression (Chronic RT effect)

Results demonstrated significant changes in gene expression for Pax-7 (RT-CON: $p = 0.0151$; RT-VAR: $p = 0.0032$), MyoD (RT-CON: $p = 0.0181$; RT-VAR: $p = 0.0107$), MMP-2 (RT-CON: $p = 0.0363$; RT-VAR: $p = 0.0318$), and COL3A1 (RT-CON: $p = 0.0030$; RT-VAR: $p < 0.0002$) (Table 2). No significant changes in MGF, MyoG, and MMP-9 mRNA expression ($p > 0.05$) were found (Table 2). Importantly, no significant differences were found between RT-CON and RT-VAR for any target gene ($p > 0.05$) (Table 2).

TABLE 1 Type I and II muscle fiber distribution, cross-sectional area (fCSA), satellite cells, myonuclear number, and domain size at baseline (Pre) and after 8 weeks (Post) of control resistance training (RT-CON) and variable resistance training (RT-VAR)

			Post	
Variables	**Fiber type**	**Pre**	**RT-CON**	**RT-VAR**
Fiber distribution (%)	I	41.1 ± 11.3	42.8 ± 12.8	42.0 ± 14.1
	II	58.9 ± 11.3	57.2 ± 12.8	58.0 ± 14.1
fCSA per fiber (μm^2)	I	5617.62 ± 1111.88	6348.71 ± 972.21*	6311.07 ± 1035.77*
	II	6323.63 ± 1291.28	7124.91 ± 1367.20*	7094.78 ± 1427.28*
Satellite cells per fiber (n)	I	0.077 ± 0.032	0.066 ± 0.045	0.075 ± 0.035
	II	0.073 ± 0.026	0.063 ± 0.054	0.069 ± 0.037
Myonuclei number per fiber (n)	I	4.78 ± 0.97	4.65 ± 0.72	4.47 ± 0.72
	II	4.03 ± 0.89	3.65 ± 0.76	3.67 ± 0.90
Myonuclear domain size per fiber (μm^2)	I	1195.80 ± 214.96	1380.06 ± 206.16*	1434.22 ± 265.97*
	II	1607.84 ± 341.17	1978.92 ± 325.57*	1997.51 ± 460.16*

Note: Values are expressed as mean ± SD. *Significantly different from Pre ($p < 0.05$).

3.4 | mRNA gene expression (Acute RT effect)

No significant differences in acute gene expression were detected between RT-CON and RT-VAR at baseline (0 h) for all assessed genes ($p > 0.05$) (Table 3). Both RT-CON and RT-VAR significantly increased MGF (main time effect; $p = 0.0060$), Pax-7 (main time effect, $p < 0.0001$), and MMP-9 (main time effect; $p = 0.0199$) mRNA expression from 0 to 24 h (i.e., acute). No significant differences were found between RT-CON and RT-VAR at 24 h for MGF, Pax-7, and MMP-9 ($p > 0.05$). For MyoG, only RT-VAR presented significant increase from 0 to 24 h (time vs protocol interaction; $p < 0.0001$). Additionally, RT-VAR was significantly different from RT-CON at 24 h (time vs protocol interaction; $p < 0.0261$). MyoD, MMP-2, and COL3A1did not change from 0 to 24 h ($p > 0.05$) (Table 3).

TABLE 2 Relative chronic gene expression at baseline (Pre) and after 8 weeks (Post) for control resistance training (RT-CON) and variable resistance training (RT-VAR)

		Post	
Gene	**Pre**	**RT-CON**	**RT-VAR**
MGF	1.14 ± 0.65	1.23 ± 0.86	1.61 ± 1.12
Pax-7	0.98 ± 0.50	1.65 ± 0.83*	2.17 ± 1.11*
MyoD	1.70 ± 0.78	4.13 ± 3.48*	3.86 ± 3.06*
MyoG	1.08 ± 0.55	0.90 ± 0.24	0.81 ± 0.37
MMP-2	1.10 ± 0.59	1.57 ± 0.70*	1.88 ± 1.16*
MMP-9	1.00 ± 0.57	1.35 ± 1.52	1.37 ± 1.50
COL3A1	1.12 ± 0.56	3.42 ± 2.61*	3.82 ± 2.16*

Note: Values are expressed as mean ±DP. *Significantly different from Pre ($p < 0.05$).

3.5 | Correlation analysis

Significant correlations were found for changes in total fCSA ($p < 0.0001$; $r = 0.89$), total SC ($p < 0.0001$; $r = 0.82$) and a strong trend for total myonuclei ($p < 0.07$; $r = 0.42$) between RT-CON and RT-VAR legs. However, no correlations were observed for chronic and acute gene expression between RT-CON and RT-VAR legs ($p > 0.05$), except for chronic MyoD expression ($p < 0.0001$; $r = 0.87$).

The collapsed analysis (RT-CON + RT-VAR) found significant correlations between total fCSA changes with total SC changes ($p < 0.01$; $r = 0.42$) and fCSA changes with total myonuclei changes ($p < 0.001$; $r = 0.50$) changes. However, no significant correlation was observed between total fCSA changes and gene expression (chronic and acute) ($p > 0.05$), except for chronic ($p < 0.001$; $r = 0.56$) and acute ($p < 0.02$; $r = 0.43$) MyoD expression.

3.6 | Heatmaps

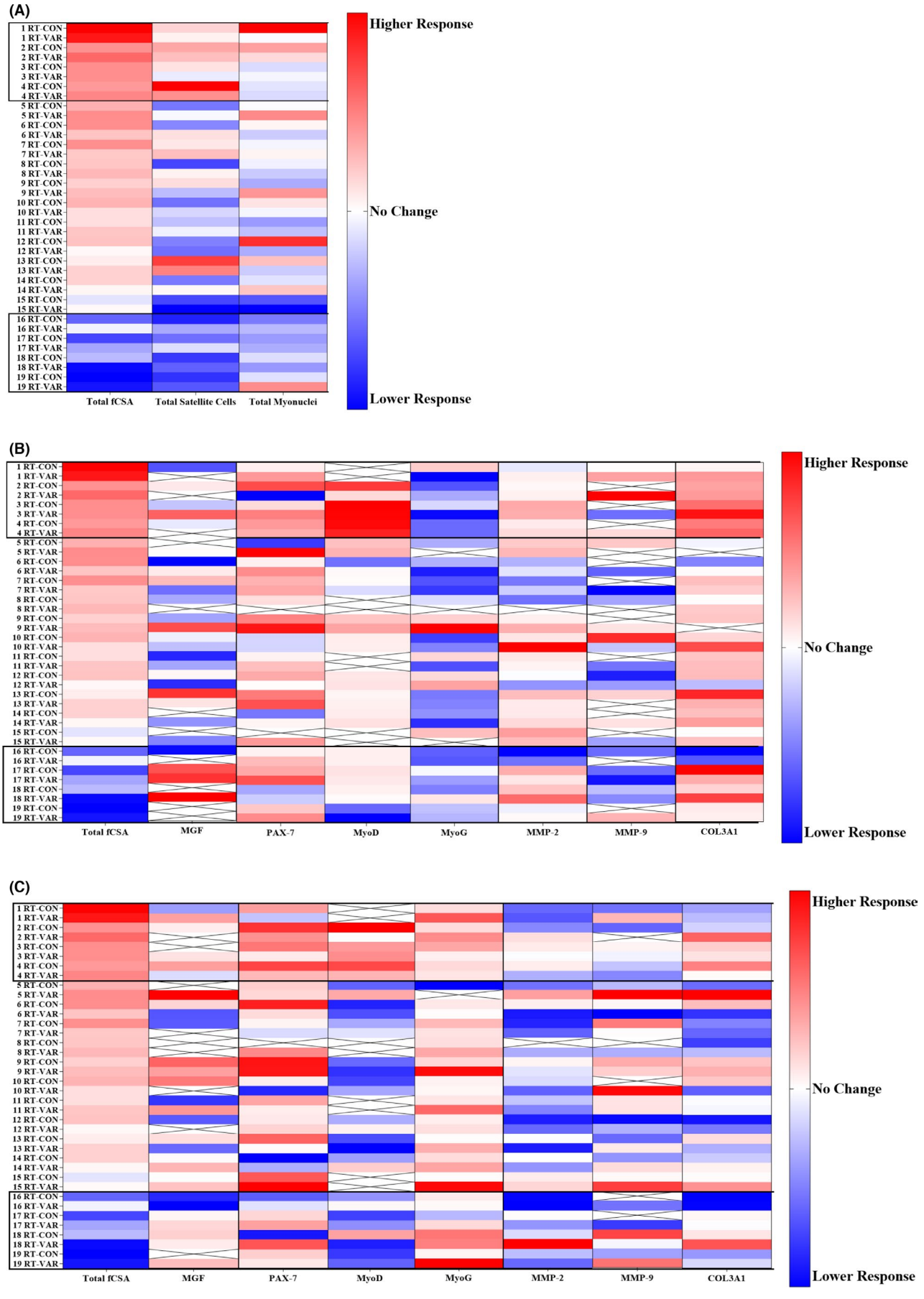
Heatmaps show a coupled response between the magnitude of total fCSA increases with SC and myonuclei responses (Δ%). This is highlighted by the higher and lower responders for total fCSA who also presented the highest and lowest SC and myonuclei responses, respectively (Figure 2A). Additionally, the heatmaps showed that both legs of each subject were in similar color shades, indicating

TABLE 3 Relative acute gene expression at baseline (0 h) and after (24 h) training sessions for control resistance training (RT-CON) and variable resistance training (RT-VAR)

	RT-CON		RT-VAR	
Gene	**0 h**	**24 h**	**0 h**	**24 h**
MGF	1.24 ± 0.88	1.96 ± 1.36*	1.23 ± 0.85	2.46 ± 1.77*
Pax-7	1.17 ± 0.62	2.12 ± 1.00*	1.22 ± 0.62	1.88 ± 0.91*
MyoD	0.91 ± 0.77	1.10 ± 1.05	0.84 ± 0.67	0.96 ± 0.62
MyoG	1.03 ± 0.28	2.12 ± 1.20	1.03 ± 0.27	4.25 ± 3.03*,†
MMP-2	1.12 ± 0.52	0.93 ± 0.33	1.01 ± 0.63	1.09 ± 1.06
MMP-9	1.50 ± 1.68	2.24 ± 2.48*	1.30 ± 1.39	3.37 ± 3.76*
COL3A1	1.35 ± 1.04	1.43 ± 0.86	1.23 ± 0.74	1.79 ± 1.52

Note: Values are expressed as mean ± DP. *Significantly different from 0 h ($p < 0.05$). †Significantly different from 24 h ($p < 0.0261$).

FIGURE 2 Heatmap of the magnitude of change (Δ%) for each dependent variable. (A) total fCSA Δ% (Pre to Post), total SC Δ% (Pre to Post), and total myonuclei content Δ% (Pre to Post). (B) total fCSA Δ% (Pre to Post) and chronic gene expression Δ% (Pre to Post). (C) total fCSA Δ% (Pre to Post) and acute gene expression Δ% (0 h to 24 h). The magnitude of change for each variable is indicated by the color gradient. The most intense shades of red in the red gradient represent the greatest magnitudes of change for the dependent variable. As the color gradient becomes whiter, it indicates no change in the dependent variable (i.e., Δ% = 0). As the color gradient flows from white to more intense shades of blue, it indicates a decrease (i.e., negative Δ%) in the values of the dependent variable. Cells filled with "X" represent missing data


(A)
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Total Satellite Cells
Total Myonuclei
Higher Response
No Change
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(B)
Total fCSA
MGF
PAX-7
MyoD
MyoG
MMP-2
MMP-9
COL3A1
Higher Response
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COL3A1
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No Change
Lower Response

that the magnitude of individual responses for total fCSA, SC, and myonuclei was not substantially modulated by a specific protocol (RT-CON or RT-VAR) (Figure 2A). On the contrary, we showed a poor alignment between the individual magnitude of total fCSA increases with chronic (Figure 2B) or acute (Figure 2C) gene expression. In this regard, the heatmaps illustrated that some of the highest responders for total fCSA presented the lowest responses for chronic and/or acute gene expression. In contrast, some of the lowest responders for total fCSA showed the highest chronic and/or acute gene expression (Figure 2B,C). Furthermore, while the gene expression was not differently affected by the RT protocols for some subjects (e.g., subjects 4 and 19 [chronic MyoD expression]—Figure 2B; subjects 3 and 16 [acute COL3A1 expression]—Figure 2C), others presented higher responses for a specific protocol (e.g., RT-CON leg) and lower responses for the other protocol (e.g., RT-VAR leg) in the heatmaps (e.g., subjects 2 and 5 [chronic Pax-7 expression]—Figure 2B; subject 18 [acute PAX-7 expression] Figure 2C).

4 | DISCUSSION

We demonstrated that frequent manipulations of RT variables do not enhance fCSA increase compared to a standard RT protocol using a within-subject design in resistance-trained subjects. Additionally, we did not find increases in SC and myonuclei content either for RT-CON and RT-VAR, with the gene expression suggesting a SC self-renewal for both protocols. The individual analyses demonstrated, to some extent, an aligned response between fCSA, SC, and myonuclei responses (i.e., subjects who had greater muscle fiber hypertrophy also had a greater SC and myonuclei responses, and the opposite is true), regardless of the RT protocol. Additionally, we did not find an alignment between chronic or acute increases in gene expression and fCSA changes. Finally, our histological assays suggest that individual SC and myonuclei responses exert a more pronounced effect on fCSA increases than extrinsic manipulations of RT variables.

4.1 | Effects of resistance training variables manipulations at group level

To optimize/maximize muscle hypertrophy and/or potentiate the hypertrophy of specific muscle fiber types, reputable guidelines advise frequent manipulation in RT variables, especially for resistance-trained individuals.[2,4,5,9] However, we found similar increases in fCSA for both type I and II fibers between protocols, regardless the slight difference in accumulated volume load between legs, confirming our hypothesis. Other authors had also demonstrated similar increases in both fiber types regardless of the RT load (low vs. high load)[27] or differences in accumulated load volume[28,29] when both protocols were performed to concentric muscle failure in resistance-trained subjects. Additionally, these results corroborate with our previous study that demonstrated similar increases in the vastus lateralis cross-sectional area for RT-CON and RT-VAR in the same cohort of resistance-trained young men.[16] Nevertheless, we expand our previous results demonstrating that frequent manipulation of RT variables (i.e., load, volume, muscle action, and rest) do not enhance muscle hypertrophy response at the fiber level, at least when RT is performed to, or near to, concentric muscle failure.

The SC response is an intrinsic mechanism suggested to involve in the RT-induced muscle hypertrophy.[12-14,18] It is suggested that this mechanism is important during fCSA increases.[12,14,19,30] However, the fCSA increases reported herein were not accompanied by chronic (i.e., basal pre-to-post changes) increases in the number of SC and myonuclei after 16 RT sessions. Despite the paucity of studies investigating the chronic SC response in previously resistance-trained individuals, the evidence demonstrated that for novice RT practitioners, the extensive increase in the fCSA in the early stages of RT induces a proliferation of SC pool to support the myonuclei donation to muscle fibers, undergoing sarcoplasmic volume expansion.[12,14,19] However, we did not report increases in SC or myonuclei content herein. Importantly, our recent meta-analysis demonstrated that a substantial SC-mediated myonuclei addition is observed when fCSA increases ≥22%, whereas our subjects presented ~12% of increase in fCSA.[30] It is possible that our resistance-trained participants have already experienced myonuclear addition; therefore, the pre-existing number of myonuclei was able to support the increase in sarcoplasmic volume (i.e., our subjects did not require new SC-mediated myonuclei addition). This hypothesis seems to be supported by studies that demonstrated ~2.5 myonuclei per fiber in novice RT practitioners,[12,14,31] while we showed ~4.0 myonuclei per fiber in resistance-trained subjects. Although both RT protocols did not significantly increase SC content in our resistance-trained subjects, they may have stimulated the SC self-renewal to maintain the SC pool. This hypothesis can be supported by chronic increases in Pax-7 and MyoD expression without increases in MyoG, suggesting an activation and proliferation of SC pool without subsequent differentiation.[20,32–35] Additionally, it has already been demonstrated that SC self-renewal and maintenance of the pool are influenced on the activity of MMPs to remodel the ECM.[36,37] Accordingly, we demonstrated increases in chronic MMP-2 and acute MMP-9 expression, which is associated with chronic increases in collagen expression (a proxy marker

of ECM remodeling). These results expand our previous findings,[38] suggesting that even subjects with years of RT experience have continuous ECM remodeling. Regarding acute gene expression, it is well established that a single bout of RT produces increases in MGF, Pax-7, MyoG, and MMP-9.[13,20,39] For most of the genes assessed acutely, both protocols induced similar expression responses. These results suggest that manipulating RT variables stimulates the genes involved in myogenesis and ECM remodeling similarly to a constant progressive RT. Only for MyoG, the RT-VAR protocol induced a higher gene expression than RT-CON, suggesting a higher level of terminal myoblast differentiation for RT-VAR.[33,34] However, we did not find higher increases in SC content with subsequent myonuclei addition for RT-VAR. These results support the hypothesis that although acute MyoG expression is responsive to RT manipulations,[40] these results are not accompanied by differences in muscle fiber hypertrophy.

4.2 | Effects of resistance training variables manipulations at individual level

Regarding the individual responses, we found large between-subject variability in fCSA, corroborating our previous findings at the whole-muscle level.[16] Additionally, the wide and similar between-subject variability in the fiber hypertrophy for RT-CON (−14.7% to 69.4% for fCSA type I and −14.9% to 58.5% for fCSA type II) and RT-VAR (−18.2% to 59.5% for fCSA type I and −15.8% to 65.5% for fCSA type II) expands our previous results suggesting that RT regimen (RT-CON or RT-VAR) do not impact the high between-subject variability also at the fiber level. In accordance, the heatmaps demonstrated that the RT-induced individual hypertrophic adaptations (i.e., between legs) were also not affected by the RT regimen, that is, neither the frequent manipulation of the RT variables (RT-VAR) nor the standard RT program (RT-CON) were able to modulate the individual response substantially. In this regard, the heatmap demonstrated that no subject presented substantial differences in the color gradient between conditions; but, few subjects showed a slightly variation between conditions with colors ranging from white to blue or white to red. In other words, the low variability of the hypertrophic response at the within-subject (between legs) level illustrates that the subjects did not have a greater benefit in the hypertrophic adaptations with a given protocol. This is supported by the high correlation ($p < 0.0001$; $r = 0.89$) between RT-CON and RT-VAR for total fCSA changes. Taken together, these results suggest that the influence of RT modulation (RT-VAR) at the within-subject (between legs) level is substantially smaller than the effects of individual responsiveness (between-subjects) on muscle fiber hypertrophy. Therefore, we suggest that the intrinsic individual capacity to respond to a RT program can be the key determinant of RT-induced hypertrophy rather than the extrinsic manipulation of RT variables.

Regarding the role of SC on individual muscle adaptations, many studies have investigated the SC-mediated myonuclei addition as a potential regulator of individual RT-induced hypertrophic response.[12,14] Some studies showed a relationship between the magnitude of fCSA increases with the SC response and myonuclei addition,[12,14] while others demonstrated similar RT-induced SC response[18] and myonuclei addition,[15] regardless the magnitude of the muscle hypertrophy. In those studies, it should be noted that clusters were composed of young and older subjects of both sexes without previous RT experience,[12,14] or by young novice RT practitioners.[18] Our results suggest that for a cohort of young resistance-trained subjects, the RT-induced SC and myonuclei responses are aligned with the magnitude of fCSA increases. Our heatmap demonstrated that the highest responders for total fCSA gains presented some of the highest SC and myonuclei responses. In contrast, the subjects with lowest hypertrophic response showed the lowest SC and myonuclei responses. Furthermore, the heatmap illustrates that none of the subjects with the highest increases of total fCSA presented the lowest responses for SC and or myonuclei responses, and the opposite is true. These results also agree with the significant correlation between total fCSA changes and total SC ($p < 0.01$; $r = 0.42$) and total myonuclei ($p < 0.001$; $r = 0.50$) changes. Similar to the fCSA results, the SC and myonuclei responses also seem to be little affected by the frequent manipulation of RT variables (i.e., no difference within-subject), at least when high effort RT protocols are applied. We demonstrate a high between-subjects variability with heatmaps colors gradient ranging from the most intense shades of blue to the most intense shades of red for both SC and myonuclei responses. Conversely, heatmaps demonstrated a lower within-subject (between legs) variability, with most subjects' legs ranging within the same gradient of colors and only a few subjects presenting a slight variation between colors gradient for both SC and myonuclei responses. These results align with the correlation ($p < 0.0001$; $r = 0.82$) between RT-CON and RT-VAR for total SC changes and a strong trend for total myonuclei ($p < 0.07$; $r = 0.42$) changes. These results suggest that SC and myonuclei responses to chronic RT are determinants of the individual muscle fiber hypertrophy and were not modulated by the manipulation of RT applied herein.

In contrast, we observed a non-alignment between the muscle fiber hypertrophy and the expression of most of the investigated genes in both the acute and chronic stages. The significant correlation between muscle fiber

hypertrophy and chronic and acute gene expression only for MyoD suggests that the expression of the vast majority of hypertrophy-related genes may not be good predictors of phenotypic adaptations.[41,42] Other studies demonstrated a non-alignment between muscle adaptations and acute[38,43] and chronic[18,38] changes in gene expression. However, little is known about the individual RT-induced change in acute and chronic gene expression in resistance-trained subjects. Our heatmaps showed that several subjects that presented the highest total fCSA increases demonstrated one of the lowest gene expression responses for several target genes, while subjects with the lowest fCSA response showed robust gene expressions. Additionally, some subjects presented the highest gene expression level for one leg while the opposite leg presented the lowest gene expression level, which corroborates with the lack of correlation between legs for acute and chronic gene expression, except for MyoD. Collectively, these results suggest a disconnection between most of the analyzed genes and the RT-induced phenotypic adaptation. Buccitelli and Selbach[44] suggested that although gene expression provides important insights into biological systems, the differential translation, protein degradation, contextual confounders, and pervasive protein-level buffering, difficult the statement that proteins are actually being synthesized or phenotype changes are responding proportionally to the gene expression. In this regard, gene expression should not be interpreted as the final output of phenotypic responses. Thus, acquiring and integrating multi-omics datasets provide a better understanding of the flow of information from genomic to proteomic to phenotypic level.[44,45] Furthermore, one may argue that the poor agreement between muscle fiber hypertrophy with acute and chronic gene expression is because subjects present different individual time points to increase the gene expression. Supporting this hypothesis, Nederveen et al.[21] assigned subjects in clusters accordingly to the capillary density (which is thought to support SC responses and hypertrophy) and demonstrated that the expression of myogenic genes significantly increased in different time points (i.e., 6 vs. 24 vs. 72 vs. 96 h after a RT bout) in each cluster. Although Nederveen et al.[21] had assessed the expression of myogenic genes in a wide range of time points, the authors did not investigate the responses of these genes on an individual basis. In contrast, Mallinson et al.[43] demonstrated different levels of individual mRNA expression throughout a RT program (i.e., 24 h after the 1st RT session vs. 7 days vs. 28 days vs. 84 days of the RT program) for 93 genes involved in RT-induced skeletal muscle regulation. Therefore, it is possible that some subjects characterized as low or high responders for gene expression could present a different level of responsiveness if analyzed in another time point. Finally, the lack of alignment may be related to random biological variability. Islam et al.[46] investigated the repeatability of the entire RT-PCR (from muscle biopsy to gene amplification), submitting the same subjects to two identical exercise bouts. Despite the similar gene expression results at the group level and a good to excellent repeatability in each step of RT-PCR, there was a lack of repeatability at the individual level at the basal state and between exercise bouts. These results suggest that although inferences based on exercise-induced transcriptional responses appear to be valid at the group level, they may not be a sensitive biomarker of adaptive potential and/or an indicator of individual responsiveness to a given exercise protocol. Thus, we suggest that future studies should include different time points, use techniques that encompass biological processes besides gene expression (e.g., multi-omics analyses) also on an individual basis, and be cautious when attributing solely to genes the role of the mechanisms involved in phenotypic responses, mainly at the individual level.

Our study is not without limitation. To minimize the number of muscle biopsies and reduce the burden to our subjects, we performed a unilateral biopsy only at baseline. The baseline muscle samples harvested from one leg may be a limitation in our design since a variability may exist between legs, although previous studies have not demonstrated significant/important differences for fiber cross-sectional area, myonuclei content, and gene expression between legs in a within-subject design.[47,48]

5 | CONCLUSION

Frequent manipulations of resistance training variables promoted similar responses on type I and II muscle fiber hypertrophy, satellite cells and myonuclei content, and gene expression as compared a standard resistance training program in resistance-trained subjects. Additionally, both protocols produce similar low within-subject and high between-subjects variability. Finally, the individual analyses showed that the muscle fiber hypertrophy is aligned with the satellite cell response and myonuclei responses, but not with chronic and acute gene expression, regardless of the resistance training protocol.

6 | PERSPECTIVE

The present study provides novel information about the effects of frequent manipulation of RT variables in the fCSA increase and related intrinsic biological factors. This study showed that fCSA, SC, myonuclei, and gene expression responses seem not to be modulated by RT variables manipulations. Additionally, the low within-subject and high between-subject variability suggests a higher contribution

of intrinsic biological factors in the RT-induced increases in fCSA compared with RT variables manipulations. This work suggests that although frequent RT variables manipulations could be performed by practitioners that appreciate this strategy (e.g., making the training less monotonous and/or feeling more motivated), subjects that do not tolerate these manipulations will not have impaired responses performing a standard progressive RT. Future study should investigate the effects of other training schemes (e.g., periodization models and blood flow restriction) and intrinsic biological factors (e.g., ribosome biogenesis, microRNAs, and capillary density) in the inter- and within-subjects skeletal muscle responses.

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CONFLICT OF INTEREST

Dr. Phillips reports sitting on the scientific advisory board for Enhanced Recovery but receives no payment outside the submitted work. In addition, Dr. Phillips has a patent Canadian 3052324 issued to Exerkine, and a patent US 20200230197 pending to Exerkine but reports no financial gains. No conflicts of interest, financial or otherwise, are also declared by other authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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