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# Acute Effects of Different Number of Sets and Non-Equalized Volume on Muscle Thickness, Peak Force, and Physical Performance in Recreationally-Trained Participants

Paulo H. Marchetti<sup>1</sup>, Ashley Samaniego<sup>1</sup>, Robert Talamantez<sup>1</sup>, Charles R. Lopes<sup>2</sup>, and Vincent G.F. Martinez<sup>1</sup>

<sup>1</sup>Department of Kinesiology, California State University, Northridge, CA, USA <sup>2</sup>Pontifical Catholic University of Campinas, Campinas, Brazil

#### Abstract

This study aimed to measure the acute effects of resistance training (RT) protocols with a different number of sets and non-equalized volume on muscle thickness, peak force, and physical performance in recreationally trained participants. Fifteen participants performed the unilateral biceps curl exercise in four different RT protocols (G<sub>4</sub>: 4 sets of 10RM, G<sub>8</sub>: 8 sets of 10RM, G<sub>12</sub>: 12 sets of 10RM, and G<sub>16</sub>: 16 sets of 10RM). The average number of repetitions (ANR), the total number of repetitions (TNR), time under tension (TUT), muscle thickness (MT), peak force (PF), and rating of perceived exertion (sRPE) were measured pre-test and post-test. ANOVAs were used to test differences between all dependent variables. For ANR, there were differences between  $G_4xG_8$ ,  $G_4xG_{12}$ , and  $G_4xG_{16}$ . For TNR, there were differences between all RT protocols. For TUT, there were differences between the first and last set for all RT protocols and between RT protocols for the last set. For PF, there were differences between the pre- and post-test for all RT protocols and between RT protocols for Post-0 ( $G_4 \times G_{12}$ ,  $G_4 \times G_{16}$ , and  $G_8 \times G_{16}$ ). For MT, there were differences between the pre- and post-test for all RT protocols. In conclusion,  $G_8$ ,  $G_{12}$ , and  $G_{16}$  showed lower ANR than  $G_4$ , TNR increased with increasing sets, and TUT increased in all RT protocols. PF decreased with an increasing number of sets, and all RT protocols increased MT. The sRPE was similar to RT protocols.

Keywords: neuromuscular fatigue, muscular performance, skeletal muscle

# **1** Introduction

Resistance training (RT) is an effective tool to induce acute muscle responses (e.g., cell swelling and neuromuscular fatigue) and chronic muscle adaptations (e.g., muscle hypertrophy, endurance, power, or strength) (Scarpelli et al., 2022). The manipulation of the acute RT variables, such as the total number of sets per RT session and muscle group is associated with increases in strength and cross-sectional area (Mangine et al., 2015); however, the upper and lower limit of sets is under debate in the scientific literature (Aube et al., 2020; Barbalho et al., 2020; Figueiredo et al., 2018; Krieger, 2010; Schoenfeld et al., 2019; Schoenfeld et al., 2016; Smilios et al., 2003). Most studies have assessed the weekly number of sets in chronic designs; however, understanding the acute responses from an RT session is fundamental to determining the effective volume and then the weekly sets. To the author's knowledge, no study has been conducted to assess the effective number of sets per RT session on mechanical and metabolic stress. Understanding the limits of the number of sets is essential to increase efficiency in prescribing and controlling each RT session, optimizing the time for each RT session, and inducing favorable levels of metabolic stress and mechanical tension (Lim et al., 2022; Marchetti, 2022; Medicine, 2021; Schoenfeld, 2013; Schoenfeld & Contreras, 2014).

The level of cell swelling can indirectly characterize metabolic stress after an RT exercise or workout. After an RT session, metabolic stress prod-

ucts such as growth hormone, lactate, and reactive oxygen species are produced simultaneously. They are important in activating the mammalian target of the rapamycin pathway (mTOR) and muscle protein synthesis (Hirono et al., 2020). Therefore, cell swelling immediately after an RT session may be involved in the hypertrophic metabolic stress response, resulting from increased phosphocreatine, increased lactate production and nitric oxide, accumulation of hydrogen ions, inorganic phosphate, and increase in the production of growth hormone and cortisol (Hirono et al., 2020). Specific ultrasound images can evaluate acute cell swelling via muscle thickness (MT) based on the distance from the subcutaneous adipose tissue muscle to the muscle-bone interface. MT is an image evaluation technique widely used in RT to assess the degree of cell swelling due to the workout performed (Wong et al., 2020; Yitzchaki et al., 2019). In combination with MT, the force production measured after an RT session complements the metabolic analysis as it indirectly indicates an increase in the production of lactate and hydrogen ions (neuromuscular fatigue) and may influence water uptake into muscle cells according to cell permeability (Behrens et al., 2023; Chen et al., 1996; Hirono et al., 2020; Schoenfeld & Contreras, 2014; Sjøgaard et al., 1985; Usher-Smith et al., 2009). Additionally, mechanical tension induced by an RT session causes mechanochemically transduced molecular and cellular responses in myofibers and satellite cells (Lim et al., 2022; Schoenfeld, 2010), which are fundamental to inducing acute responses and chronic adaptations such as muscle growth (Lim et al., 2022).

Additionally, studies have reported similar chronic adaptations when the total volume was equated (Figueiredo et al., 2018; Schoenfeld et al., 2014); however, in many cases, the total volume cannot be equalized for practical training reasons. So, in the present study, the total volume was not equalized, aiming to assess the acute responses between RT protocols with a different number of sets but the same external load (10RM). This study can help practitioners and strength athletes who aim to plan their training volume but do not necessarily manage to equalize the total volume. To the author's knowledge, no study has been conducted to assess the acute metabolic stress and mechanical tension induced by a different number of sets without equalizing volume in recreationally trained participants. This study intended to assess the difference between RT protocols and provide useful information to practitioners regarding the effects of

more or fewer sets in an RT session. Therefore, the main purpose of this study was to measure the acute effects of RT protocols with a different number of sets and non-equalized volume on muscle thickness, peak force, and physical performance in recreationally-trained participants. The first hypothesis was that more sets per RT protocol will induce a greater reduction in the average number of repetitions and increase the time under tension and the total number of repetitions; however, sRPE will remain constant for all RT protocols. The second hypothesis considers that more sets per RT protocol will induce a greater reduction in peak force and increase muscle thickness (Damas et al., 2018; Schoenfeld, 2010, 2013; Schoenfeld & Contreras, 2014; Schoenfeld et al., 2016). The results of this study will help coaches and trainers understand and prescribe better acute RT sessions based on the number of sets, avoiding excessive volume.

# 2 Methods

# 2.1 Participants

A pilot study determined the number of participants conducted previously based on an effect size difference of 0.95, a significance level of 5%, and a power of 80% derived from the muscle thickness of individuals with the same characteristics used in the present study. Fifteen resistance-trained men were assigned to this study [age  $25.8 \pm 2.5$  years, total body mass  $84.7 \pm 11.8$  kg, height  $176.7 \pm 4.9$ cm, dominant biceps curl exercise (10RM) 12.3  $\pm$ 2.9 kg, non-dominant biceps curl exercise (10RM)  $11.5 \pm 2.6$  kg]. All participants were familiar with hypertrophy-type training and had regularly engaged in an RT routine for more than a year. Additionally, they were familiar with the standing unilateral biceps curl exercise. They had 3±1 years of resistance training experience (at least 3 times a week), with no previous surgery or history of injury with residual symptoms (pain) in the upper limbs or spine within the last year. The Institutional Review Board (IRB) approved this study (00001788/2018). The participants were informed of the risks and benefits of the study before any data collection and then read and signed an institutionally approved informed consent document.

#### 2.2 Procedures

This study used a randomized and counterbalanced design. Participants attended three laboratory sessions and refrained from performing upperbody exercises other than activities of daily living for at least 48 hours before testing. A withinparticipant approach was used in which each participant performed all RT protocols. Each RT protocol was performed unilaterally, and all sets were performed until concentric muscular failure (RM). The RT protocols were defined as follows:  $G_4$ : 4 sets of 10RM,  $G_8$ : 8 sets of 10RM,  $G_{12}$ : 12 sets of 10RM, and  $G_{16}$ : 16 sets of 10RM.

For the first session, participants were asked to identify their preferred arm for writing, which was considered their dominant arm (Maulder & Cronin, 2005). Then, anthropometric data (height, weight, and upper limb length) were evaluated. Next, all participants performed a familiarization and specific warm-up for the unilateral biceps curl exercise. The warm-up followed the following procedure: 1 set of 15 repetitions without external load, followed by 1 set of 10 repetitions with 5kg for each exercise, and 5-minute rest intervals were given between sets. To perform the unilateral biceps curl exercise, all participants stood before the cable pulley machine with a supinated grip on a handle. Any trunk movement was avoided during the protocols by the researcher in charge. They lifted the weight stack from complete elbow extension to complete elbow flexion (concentric phase) and then returned to a full elbow extension (eccentric phase). Then, a 10RM (repetitions maximum) testing was applied to both upper limbs in random order. The 10RM testing was based on the National Strength and Conditioning Association (NSCA) guidelines to determine individual initial training loads (Haff & Triplett, 2016). Attempts were performed to progressively increase the external loads until they reached the maximal capacity to perform 10RM with the correct technique. The movement velocity was selfselected.

For the second and third sessions, the participant's arms were randomly allocated within one specific RT protocol and sequence (RT protocol vs. dominant or non-dominant arm). Each participant performed two RT protocols per session, one for each arm. A specific warm-up (unilateral biceps curl exercise) was conducted during each session with 10 repetitions at 50% of their 10RM testing load. Then, as a pre-test, an ultrasound imaging of the elbow flexors was carried out followed by the maximal voluntary isometric force testing at 90 degrees of elbow flexion. Then, participants performed one of four RT protocols in random order ( $G_4$ : 4 sets of 10RM/2-min rest,  $G_8$ : 8 sets of 10RM/2-min

rest, G<sub>12</sub>: 12 sets of 10RM/2-min rest, and G<sub>16</sub>: 16 sets of 10RM/2-min rest). After each RT protocol, the ultrasound image of the elbow flexors and the maximal voluntary isometric force were retested immediately after (post-0), 15-min after (post-15), and 30-min after (post-30) the end of each session (Aleais et al., 2022; Marchetti et al., 2020; Smith et al., 2021). All participants reported a rating of perceived exertion (RPE) 30-min after each RT protocol and session. The cadence (velocity) was selfselected. In the same session, 60-min after the first RT protocol with one arm, all measures were carried out on the contralateral arm and the participants performed another RT protocol. All four RT protocols were performed at the end of the two sessions, with two RT protocols for each arm. So, two RT protocols were performed in the same session because there was no influence between arms for all variables analyzed as observed in the pilot study and other studies carried out by the same laboratory (Marchetti et al., 2020). All tests were directly supervised by a research assistant (CSCS certified) to ensure proper performance and correct technique. All participants received similar verbal encouragement during all RT protocols, and all measures were performed at the same hour of the day (between 1 PM and 4 PM) by the same researcher.

# 2.3 Measurements

## 2.3.1 Total Number of Repetitions (TNR)

The TNR was defined by the sum of the number of repetitions in each set for each RT protocol.

## 2.3.2 Average Number of Repetitions (ANR)

The ANR was calculated by dividing TNR per number of sets for each RT protocol.

## 2.3.3 Time Under Tension (TUT)

A chronometer measured the TUT during each set for all RT protocols. Then, to define the TUT, the set duration in seconds was divided by the maximal number of repetitions. TUT was calculated for the first and last set for further analysis.

# 2.3.4 Session Rating of Perceived Exertion (sRPE)

The session RPE was assessed with a CR-10 scale using the recommendations of Sweet et al., (Sweet

et al., 2004). Participants were asked to use an arbitrary unit (A.U.) on the scale to rate their overall effort after all RT protocols. A rating of 0 was associated with no effort, and a rating of 10 was associated with maximal effort and the most stressful exercise ever performed. All participants answered the following question based on CR-10 scale: "How was your workout?" The sRPE was asked 15-min after the end of each RT protocol.

#### 2.3.5 Peak Force (PF)

The PF was measured by a digital load cell acquisition system (FM-204-1000K, Shenzhen Aermanda Technology Co. Ltd., Shenzhen, Guangdong, China / Capacity: 1000Kgf / Resolution: 0.01kgf). To perform the maximal isometric force testing, all participants stood before the cablepulley machine with a supinated grip on a handle. All participants performed 3 maximal voluntary isometric contractions (MVIC) at 90 degrees of elbow flexion before (pre-test), immediately after (post-0), 15-min after (post-15), and 30-min (post-30) each RT protocol (Marchetti et al., 2020; Smith et al., 2021). Each MVIC was performed for 5-sec and 10-sec rest intervals. The peak force (PF) of each MVIC was defined, and the average of the 3 MVICs was used for further analysis. The testretest ICC (PF) was 0.95.

#### 2.3.6 Muscle Thickness (MT)

Ultrasound imaging was used to obtain measurements of MT. A trained technician performed all testing using an ultrasound imaging portable unit (Hitachi Noblus; Hitachi Medical Corporation, Tokyo, Japan). Following a generous application of a water-soluble transmission gel (Cskin, Medics Medical Products LLC., NY, USA) to the measured site, a 7.5-MHz linear array probe (L55 Probe) was placed perpendicular to the tissue interface without depressing the skin. Equipment settings were optimized for image quality according to the manufacturer's user manual and held constant in all sessions. When the quality of the image was deemed to be satisfactory, the image was saved to the hard drive. The MT dimensions were obtained by measuring the distance from the subcutaneous adipose tissue-muscle interface to the muscle-bone interface per methods (Abe et al., 2014). Measurements were taken on both sides of the body at the elbow flexors. The upper arm measurements were conducted while participants were in a standing position. For the elbow flexors, measurements were taken at 60% distal between the humerus's lateral epicondyle and the scapula's acromion process. To maintain consistency between pre- and post-test, each site was marked with ink. To further ensure the accuracy of measurements, at least 3 images were obtained for each side. A fourth image was obtained and averaged if measurements were more than 1mm from one another. MT was measured before (pre-test), immediately after (post-0), 15-min after (post-15), and 30-min (post-30) each RT protocol. The test-retest ICC (MT) was 0.96-0.98, and the intra-rater reliability was 0.96-0.97.

#### 2.4 Statistical Analyses

The normality and homogeneity of variances were confirmed by the Shapiro-Wilk and Levene's tests, respectively. The mean, standard deviation (SD), and delta percentage ( $\Delta$ %) were calculated. An independent sample t-test was used to compare the maximal intensity (10RM) between arms (dominant vs. non-dominant). One-way ANOVAs were used to test differences between RT protocols for ANR and sRPE. Repeated measures ANOVA (4x2)was used to test differences between RT protocols  $(G_4, G_8, G_{12}, and G_{16})$  and time (first set and last set) for TUT. Repeated measures ANOVA (4x4) were used to test differences between RT protocols (G<sub>4</sub>,  $G_8$ ,  $G_{12}$ , and  $G_{16}$ ) and time (pre-test, post-0, post-15, and post-30) for MT and PF. Post-hoc comparisons were performed with the Bonferroni test when necessary. Furthermore, the magnitudes of the difference were examined using the standardized difference based on Cohen's d units using effect sizes (d) (14). The d results were qualitatively interpreted using the following thresholds: <0.35 trivial; 0.35-0.8 - small; 0.8-1.5 - moderate; >1.5 large for recreationally trained (Cohen, 1988). An alpha of 5% was used to determine statistical significance.

# **3** Results

For the 10RM testing, there was no significant difference between dominant and non-dominant arms (12.3  $\pm$  2.9 kg x 11.5  $\pm$  2.6 kg, respectively,  $\Delta$ %=6.5, p>0.05).

For the average number of repetitions (ANR) (Figure 1a), there were observed statistical differences between RT protocols:  $G_4 \times G_8$  (p=0.030, d=1.17 (moderate), and  $\Delta$ %=18.3),  $G_4 \times G_{12}$  (p<0.001,

d=2.26 (large), and  $\Delta$ %=30.5), G<sub>4</sub> x G<sub>16</sub> (p<0.001, d=2.13 (large), and  $\Delta$ %=31.7). The total number of repetitions (TNR) (Figure 1b), there were observed statistical differences between RT protocols: G<sub>4</sub> x G<sub>8</sub> (p=0.001, d=2.39 (large), and  $\Delta$ %=39.6), G<sub>4</sub> x G<sub>12</sub> (p<0.001, d=3.58 (large), and  $\Delta$ %=53.3), G<sub>4</sub> x G<sub>16</sub> (p<0.001, d=3.50 (large), and  $\Delta$ %=63.7), G<sub>8</sub> x G<sub>12</sub> (p=0.030, d=1.12 (moderate), and  $\Delta$ %=22.7), G<sub>8</sub> x G<sub>16</sub> (p<0.001, d=1.97 (large), and  $\Delta$ %=39.9), G<sub>12</sub> x G<sub>16</sub> (p<0.001, d=1.06 (moderate), and  $\Delta$ %=22.3).

For time under tension (TUT) (Figure 1c), there was a significant main effect for RT protocol (p<0.001) and time (p<0.001). There was a significant interaction between RT protocol and time (p=0.035). There were significant differences between the first and last set for  $G_4$  (p<0.001, d=2.10 (large),  $\Delta$ %=32.2), G<sub>8</sub> (p<0.001, d=2.60 (large),  $\Delta$ %=41.2),  $G_{12}$  (p=0.008, d=1.41 (large),  $\Delta$ %=20.8), and  $G_{16}$ (p<0.001, d=2.16 (large),  $\Delta$ %=43.2). There were significant differences between RT protocols for the last set:  $G_4 \times G_{12}$  (p=0.003, d=1.68 (large),  $\Delta$ %=25.0), G<sub>8</sub> x G<sub>12</sub> (p=0.010, d=1.85 (large),  $\Delta$ %=29.4), G<sub>12</sub> x G<sub>16</sub> (p=0.020, d=1.76 (large)  $\Delta$ %=8.1). For Session RPE (sRPE), there was observed no statistical difference between RT protocols:  $G_4$  (8.3±1.5A.U.),  $G_8$  (9.0±1.0A.U.),  $G_{12}$  $(9.1\pm1.0A.U.)$ , and  $G_{16}$   $(9.4\pm1.1A.U.)$ .

For Peak Force (PF) (Figure 2a), there were significant main effects for RT protocol (p<0.001) and time (p<0.001). There was a significant interaction between RT protocol and time (p<0.001). There were observed statistical differences for RT protocols: G<sub>4</sub>: Pre-test x Post-0 (p<0.001, d=0.89 (moderate), and  $\Delta$ %=19.4), Pre-test x Post-30 (p=0.001, d=0.45 (small), and  $\Delta$ %=10.3); G<sub>8</sub>: Pre-test x Post-0 (p<0.001, d=0.95 (moderate), and  $\Delta$ %=22.9), and Pre-test x Post-15 (p=0.008, d=0.93 (moderate), and  $\Delta$ %=11); G<sub>12</sub>: Pre-test x Post-0 (p<0.001, d=1.32 (moderate), and  $\Delta$ %=30.7), Pre-test x Post-15 (p=0.014, d=0.71 (small), and  $\Delta$ %=17.7); G<sub>16</sub>: Pre-test x Post-0 (p<0.001, d=2.21 (large), and  $\Delta$ %=40.0), Pre-test x Post-15 (p=0.001, d=1.03) (moderate), and  $\Delta$ %=20.7), and Pre-test x Post-30 (p=0.001, d=0.93 (moderate), and  $\Delta$ %=18.8). There were observed statistical differences for Post-0 between RT protocols: G<sub>4</sub> x G<sub>12</sub> (p=0.026, d=0.81 (moderate), and  $\Delta$ %=18.8), and G<sub>4</sub> x G<sub>16</sub> (p=0.003, d=1.45 (moderate), and  $\Delta$ %=29.0), and G<sub>8</sub> x G<sub>16</sub> (p=0.050, d=0.95 (moderate), and  $\Delta$ %=21.3).

For muscle thickness (MT) (Figure 2b), there was a significant main effect only for time (p=0.015). There was no significant interaction between RT

protocol and time (p=0.053). There were observed statistical differences for RT protocols:  $G_4$ : Pre-test x Post-0 (p<0.001, d=1.42 (moderate), and  $\Delta$ %=12.5), Pre-test x Post-15 (p=0.001, d=0.81 (moderate), and  $\Delta$ %=8.1), and Pre-test x Post-30 (p=0.007, d=0.57 (small), and  $\Delta$ %=5.8);  $G_8$ : Pre-test x Post-0 (p<0.001, d=1.63 (large), and  $\Delta$ %=13.1), and Pre-test x Post-15 (p<0.001, d=1.06 (moderate), and  $\Delta$ %=10.2); G<sub>12</sub>: Pre-test x Post-0 (p<0.001, d=1.62 (large), and  $\Delta$ %=14.3), Pre-test x Post-15 (p < 0.001, d=1.12 (moderate), and  $\Delta$ %=11.4); and Pre-test x Post-30 (p<0.001, d=0.82 (moderate),  $\Delta$ %=8.2); G<sub>16</sub>: Pre-test x Post-0 (p<0.001, d=1.47 (moderate), and  $\Delta$ %=14.7), Pretest x Post-15 (p<0.001, d=1.15 (moderate), and  $\Delta$ %=11.8), and Pre-test x Post-30 (p<0.001, d=0.90) (moderate), and  $\Delta$ %=9.95).

# **4** Discussion

This study aimed to evaluate the acute effects of RT protocols with a different number of sets and non-equalized volume on muscle thickness, peak force, and physical performance in recreationally-trained participants. The main findings include: 1) RT protocols ( $G_8$ ,  $G_{12}$ , and  $G_{16}$ ) presented a similar average number of repetitions (ANR); 2) TNR increased with an increasing number of sets; 3) TUT increased between the first and last set for all RT protocols; 4)  $G_{12}$  presented a greater increase in TUT compared to  $G_4$ ,  $G_8$ , and  $G_{16}$ ; 5) sRPE was similar between RT protocols; 6) The increase in the number of sets induced greater PF reduction; 7) All RT protocols induced similar increases in MT.

Regarding the acute variables analyzed (MNR, ANR, and TUT), the results of this study showed that the TNR increased with an increasing number of sets ( $G_{16}>G_{12}>G_8>G_4$ ); however, the ANR was similar between RT protocols ( $G_8$ ,  $G_{12}$ , and  $G_{16}$ ) with exception of  $G_4$ . The TUT increased between the first and last set for all RT protocols, however,  $G_{12}$  presented a greater increase in TUT compared to  $G_4$ ,  $G_8$ , and  $G_{16}$ . It was hypothesized that more sets per RT protocol will induce a greater reduction in ANR and increase in TUT and TNR; the results of this study partially corroborated the hypothesis because ANR was not affected by the number of sets in each RT protocol with the exception of  $G_4$ .

It is well known that the total number of sets and repetitions associated with the external load are important components of the RT program to induce acute responses and, possibly, chronic adap-



**Figure 1:** Mean  $\pm$  standard deviation of (a) the average number of repetitions, (b) the total number of repetitions, and (b) time under tension for all RT protocols.

*Note*: <sup>(a)</sup>Significant difference with  $G_4$ , p<0.05. \*Significant difference between RT protocols, p<0.05. <sup>&</sup>Significant difference between first and last set for all RT protocols, p<0.001. <sup>#</sup>Significant difference between  $G_{12}$  vs.  $G_4$ ,  $G_8$ ,  $G_{16}$ , p<0.001.



Figure 2: Mean  $\pm$  standard deviation of (a) peak force and (b) muscle thickness of elbow flexors for all RT protocols

*Note*: <sup>\$</sup>Significant difference with Pre-test, p<0.001. <sup>+</sup>Significant difference with pre-test for G<sub>8</sub>, G<sub>12</sub>, and G<sub>16</sub>, p<0.05. <sup>#</sup>Significant difference with pre-test for G<sub>4</sub> and G<sub>16</sub>, p<0.001. <sup>@</sup>Significant difference between G<sub>4</sub> x G<sub>12</sub>, G<sub>4</sub> x G<sub>16</sub>, G<sub>8</sub> x G<sub>16</sub>, p<0.001. <sup>\*</sup>Significant difference with Pre-test, p<0.001.

tations such as hypertrophy. In this study, the intensity was defined by 10RM and was kept constant during all sets and RT protocols, therefore, all variations in the acute variables were related to RT volume represented by the TNR, ANR, and TUT. In this study, the TNR for  $G_{16}$  presented the highest value when compared to  $G_{12}$ ,  $G_8$ , and  $G_4$ (22.3%, 39.9%, and 63.7%, respectively). However, the TUT for  $G_{12}$  presented the highest variation between pre- and post-test when compared to  $G_{16}$ ,  $G_8$ , and  $G_4$  (8.1%, 29.4, and 25%, respectively), representing a greater time in contraction for  $G_{12}$ . TUT has been shown to alter neurophysiological, hormonal, and metabolic responses (Burd et al., 2012; Cintineo et al., 2018; Lacerda et al., 2016; Marchetti & Lopes, 2018) and could interfere with the metabolic stress induced by each RT protocol. Extrapolating these results to chronic adaptations, TUT ranging from 0.5- to 8-sec seems to maximize muscle hypertrophy (Schoenfeld et al., 2015). So, TNR, ANR, and TUT were affected by the number of sets and it is known that high volumes might influence the dose-response relationship resulting in additional improvements in muscle mass (Schoenfeld et al., 2016). Finally, the ANR was similar between  $G_{16}$ ,  $G_{12}$ , and  $G_8$  (5.6 repetitions, 5.8 repetitions, and 6.7 repetitions, respectively).

It is well known that different RT protocols have been shown to induce different acute cell swelling, the extent of which relies on the type of exercise, level of fatigue, volume, and intensity (Schoenfeld, 2013). RT exercises with momentary muscle failure reduce the intramuscular ATP and CP levels (and Pi, ADP, and AMP accumulation) and increase the glycolytic flux (production of H+ leads to metabolite accumulation), hypoxia (via muscle contraction), and venous pooling leading to cell swelling (Chen et al., 1996; Schoenfeld & Contreras, 2014; Sjøgaard et al., 1985; Usher-Smith et al., 2009). In this study, both neuromuscular fatigue and cell swelling were assessed via PF and MT, respectively.

The peak force (PF) was measured before and after (0-min, 15-min, and 30-min) aiming to determine the level of neuromuscular fatigue induced by each RT protocol. Neuromuscular fatigue is defined as a reduction in maximal force or power production in response to contractile activity (Behrens et al., 2023). Considering the increase in the number of sets in each RT protocol, it was hypothesized that more sets per RT protocol will induce a greater reduction in PF and the results of this study partially corroborate the main hypothesis. The reduction in PF was different for all RT protocols immediately after (post-0) the training and with protocols with more sets inducing more neuromuscular fatigue  $[G_{16} (40\%) > G_{12} (30.7\%) > G_8 (22.9\%) < G_{16}$ (19.4%)].

Ultrasound images can evaluate acute cell swelling via muscle thickness (MT) which is based on defining the distance from the subcutaneous adipose tissue-muscle to muscle-bone interface for a specific muscle (Abe et al., 2014). In the present study, MT was used to measure acute cell swelling before and after (0-min, 15-min, and 30-min) all RT protocols. It was hypothesized that RT protocols with more sets per RT session will induce a greater increase in the MT response, however, the results did not corroborate the main hypothesis. All RT protocols showed a similar increase in MT immediately after training (post-0) and MT did not return to the baseline (pre-test) after 30-min rest for all RT protocols. However, it was observed that RT protocols with more sets induced small and non-significant statistical increases in MT [ $G_4$  (12.5%) <  $G_8$  (13.1%)

<  $G_{12}$  (14.3%) <  $G_{16}$  (14.7%)]. Comparing the PF and MT results, it is possible to observe that the reductions in force production did not directly represent the increases in MT. For example, after 4 sets there was a 19.4% reduction in PF with a 12.5% increase in MT, on the other hand, after 16 sets the PF reduction was 40% with a 14.7% increase in MT. So, based on these results, it is possible to hypothesize two scenarios: 1. there is a non-linear relationship between cell swelling and neuromuscular fatigue, or 2. there may be a limit of cell swelling after a certain number of sets/repetitions associated with concentric muscle failure.

Finally, the rating of perceived exertion (sRPE) is frequently used to indirectly quantify the level of effort after sets, exercises, and RT sessions (Halperin & Emanuel, 2019; Marchetti, 2022). The sRPE represents a relationship between the physiological and performance measures and assists in quantifying the overall load (Halperin & Emanuel, 2019). In this study, it was hypothesized that all RT protocols induce similar sRPE corroborating the main hypothesis. It is well known that sRPE is affected by the level of neuromuscular fatigue after RT protocols for recreationally-trained participants, however, in this study, all RT protocols presented high sRPEs (8.3-9.4 A.U.). Probably, the lack of significant difference for the sRPE scores was that the sets in all RT protocols were performed until concentric muscle failure. Therefore, even with a small (and non-significant) trend between RT protocols  $(G_{16}>G_{12}>G_8>G_4)$ , thus, it is possible that when the intensity is similar and close to muscular failure, the total number of sets does not affect the perceived exertion. Finally, the RT protocols ( $G_8$ ,  $G_{12}$ , and G<sub>16</sub>) showed lower ANR values when compared to G<sub>4</sub>, however, TNR showed increases directly related to the number of sets. The TUT increased in all RT protocols, however,  $G_{12}$  had the most significant increase when compared to  $G_4$ ,  $G_8$ , and  $G_{16}$ . PF was directly affected by progression in the number of sets, however, all RT protocols induced similar increases in MT. The sRPE was high and similar across all RT protocols. It is well known that certain levels of metabolic stress and mechanical tension are required to induce chronic adaptations such as hypertrophy. On this matter, Brigatto et al., (Brigatto et al., 2022) investigated the chronic effects (8 weeks) of 16, 24, and 32 weekly sets per muscle group on muscular strength and hypertrophy in trained men. Each muscle group was trained twice a week with 8, 12, and 16 sets per RT session per muscle group. The weekly total load lifted was higher for 32 weekly sets when compared with 24 (38.0%) and 16 (57.1%). Muscle thickness of the biceps brachii, triceps brachii, and vastus lateralis was evaluated after 8 weeks. The results showed that 32 weekly sets per muscle group presented higher values compared to 24 and 16 weekly sets per muscle group.

This study has some limitations that should be considered when interpreting the current results. First, we measured neuromuscular fatigue via force production, maybe measurements of lactate or by-products from the metabolism could improve the understanding of the metabolic stress induced by several sets. Second, the findings of this study cannot, necessarily, be generalized to other muscle groups, RT exercises, RT protocols, or different populations including adolescents, athletes, and the elderly.

# **5** Conclusion

In the present study, the highest mechanical stress was observed in the RT protocol with 16 sets since more sets and repetitions were performed with 10RM (the same external load used in all RT protocols); however, the TUT was higher in the RT protocols with 16 and 12 sets. Regarding metabolic stress, all RT protocols induced high cell swelling and reduced force production. Thus, summarizing the results, RT protocols between 12 and 16 sets may be a better option to induce high and similar levels of metabolic stress and high levels of mechanical tension.

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# **Conflict of Interest**

The authors of this study have declared that they have no conflict of interest to report.

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## ORCiD

Paulo H. Marchetti: https://orcid.org/0000-0002-2016-936X

# **Ethical Approval**

Ethical approval was obtained from the Human Research Ethics Committee of the California State University-Northridge, under protocol (00001788/2018). It was written according to the standards established by the Declaration of Helsinki.

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# **Corresponding Author**

Paulo H. Marchetti California State University, Department of Kinesiology 18111 Nordhoff Street Northridge, CA, 91330 Email: dr.pmarchetti@gmail.com Phone: +1 (818) 677-6983

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