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Original Article

The acute effect of multiple-sets and drop-set systems on the hormonal and immunological responses

RAGAMI CHAVES ALVES[1], ALYSSON ENES[2], JONATO PRESTES[3], YURI RAFAEL DIAS[4], LUIS FERNANDO COIMBRA[5], HENRIQUE LUBAS[6], FABRÍCIO BOSCOLO DEL VECCHIO[7], TÁCITO PESSOA DE SOUZA JUNIOR[8]
[1,2,8,]Research Group on Metabolism, Nutrition and Strength Training, Federal University of Paraná – PR, BRAZIL;
[3]Department of Physical Education, Catholic University of Brasilia – DF, BRAZIL;
[4,5,6,7,]Department of Physical Education, Federal University of Paraná – PR, BRAZIL;
[6] Department of Physical Education,Federal University of Pelotas – RS, BRAZIL
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Abstract:
Purpose: to compare Multiple-Set (MS) and Drop-Set (DS) systems on acute testosterone responses and at different moments for lymphocytes and monocytes in recreationally trained men. **Methods:** the study was composed of twelve male subjects(age: 22,5 ± 3,2 years; height: 176,3 ± 6,7 cm; body mass: 75,4 ± 6,9 kg) recreationally trained individuals. The participants performed the 1RM test in the Leg Press 45º and 48h post the first test, did the test again for the reproducibility of the loads. The participants were randomly for both protocols (MS: 3 x 10 reps – 75%1RM; DS: 3 x 10 reps at 80% - 40% - 20% of 1RM), which were separated by 72-hour interval between each one of them. The blood collect of testosterone levels were realized before the experimental protocols (baseline), immediately after (IP), 15-, 30- and 60-minutes after the protocols. Lymphocytes and monocytes were collected at baseline, IP, 24- and 48-hours after the experimental protocols. **Results:**MS showed an increase in the testosterone levels only at the IP and the DS showed higher values compared to MS. However, the effect size (ES) didn't showed significant differences between the different moments, both protocols presented small and trivial values. The monocytes (24 and 48 hours post-training) and lymphocytes (IP, 24 and 48 hours post-training) responses were higher in DS compared to MS, but these levels didn't exceed the reference values considered salutary. **Conclusion:** Both protocols are efficient in acute increases in testosterone levels.However, despite the higher values in testosterone levels on DS, when this values compared with MS, both protocols showed similar magnitude. In addition, the small fluctuations in lymphocytes and monocytes observed in both protocols didn't present clinical significance.
Key Words:Resistance training – testosterone – immune system – multiple-sets – drop-set..

Introduction

Resistance training (RT) is recognized as a method capable of improving health and physical fitness levels in a large part of the population (W. J. Kraemer et al., 2002; W. J. Kraemer & Ratamess, 2004). Generally, the observed improvement is determined by the configuration of the RT variables, such as intensity, volume, rest intervals between sets and exercises, exercise order, or the acute manipulation of these variables in specifically RT systems (Crewther, Cronin, & Keogh, 2005; W. J. Kraemer et al., 2002; W. J. Kraemer & Ratamess, 2004). The acute manipulation of the RT variables present different configurations, among which moderate loads can be used for heavy and low repetitions in a moderate to high number of sets per exercise (W. J. Kraemer & Ratamess, 2004). This configuration is typically used to maximize gains in muscle strength; however, moderate-to-heavy loads and multiple sets per exercise have been widely used, too, in RT for skeletal muscle hypertrophy (R. R. Kraemer et al., 2006). Thus, it is evident that the configuration of the training routine imposes a degree of stress on the organism, which initiate neural and hormonal responses that mediate long-term adjustments.

In modulating the concentration of anabolic hormones that affect muscle protein synthesis, they are largely related to the magnitude of RT stimulus (Spiering et al., 2009). Testosterone is one of these hormones, which has action increasing the rates of muscle protein synthesis and reduces the muscle protein breakdown (Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003). Thus, the greater the adrenergic stimulus provided by RT, more testosterone is secreted by the Leydig cells through the hypothalamic-pituitary-testicular axis, additionally with small amounts by the adrenals or by the conversion of other androgens (Uchida et al., 2009). This acute hormonal response is related to the hypertrophic process because the regeneration of muscle damage generated by the RT is dependent on the rates of muscle protein synthesis (Vierck et al., 2000).

Previous studies had examined changes in testosterone responses using manipulation of various RT variables, such as intensity, number of sets and repetitions, and range of rest interval between sets (Gotshalk et

Corresponding Author: RAGAMI CHAVES ALVES[1], E-mail: ragami1@hotmail.com

al., 1997; R. R. Kraemer et al., 2006; Uchida et al., 2009). The acute responses are clear, which demonstrate elevations in testosterone levels for protocols using moderate loads, high volume and short rest interval between sets. Although this state of the art is well documented in the literature, when we observe the evidence for adult individuals with greater experience (> 2 years) in the RT, there are also divergences in responses to testosterone levels. This occurs because in these individuals the load progression is determinant in order to promote the desired hypertrophic adjustments (Garber et al., 2011). In this way, studies have demonstrated the importance of the number of sets performed (i.e., total volume) allied to intensity and the reduced range of interval between sets in the regulatory process on testosterone responses (Crewther, Cronin, Keogh, & Cook, 2008). This stimulus is characterized as density, training that prioritize high volume using moderate-to-heavy loads by muscle group and reduced rest interval between sets. The question of high density training has been very propagate in the scope of hypertrophy, but little explored in relation to the extent of the inflammatory response. This could provide more comprehensive information on how this type of stimulus affects the physiology of the organism, since the inflammatory responses are also determinants for repair in muscle damage from RT session (Peake, Neubauer, Della Gatta, & Nosaka, 2016).

The acute inflammatory response is due to microtraumas in skeletal muscle fiber caused in the RT session, which is considered a temporary and repairable damage (Paulsen et al., 2005). This response results in some metabolic responses in an inflammatory pathway, initiated, among others, by neutrophils, monocytes and macrophages, whose function is the cleaning, repair and development of previously damaged tissues (Paulsen et al., 2005). Thus, we hypothesized that for well-trained individuals, the greater the inflammatory response generated by the RT session, the higher the probability of maximizing the hypertrophic responses, as well as the testosterone levels. Given this context, the proposal to increase the gains of skeletal muscle hypertrophy in trained individuals would be to apply RT sessions with density. However, until the author's knowledge, the extrapolation of the inflammatory process is not known and to what extrapolation the inflammatory adjustment generated is really benefical, or the reference values considered salutary in relation to neutrophils and monocytes are extrapoled. Additionally, if the testosterone levels increase in magnitude in proportion to the stress imposed, or if there is a plateau for that hormone response. Thus, the aim of the present study is to verify and compare Multiple-Set (MS) and Drop-Set (DS) systems on acute testosterone responses and at different moments for neutrophils and monocytes in recreationally trained men.

Material & methods

Study design

The study had a cross-sectional design. The independent variables were the MS and DS systems protocols, and the dependent variables were testosterone and lactate levels.

The participants completed four meetings with the researchers, separated by a 72-hour interval between each one of them. At the first visit, the researchers assessed the body composition; the subjects completed the Informed Consent Term (ICT), the PAR-Q questionnaire and the questions regarding the inclusion and exclusion criteria. At the second visit, the subjects were submitted to the 1 Repetition Maximum (1RM) in the Leg Press, and also received instructions on the conditions of the blood collection. After completing these procedures, in the third and fourth meeting, the experiment was started, in which the RT protocols were performed together with the lactate, testosterone, monocyte and lymphocyte collections.

Participants

The present study was composed of 12 recreationally active individuals with experience in RT program. Characterization of the subjects is show on Table 1. The participants were recruited by convenience method in training centers (gyms) of Curitiba – PR, Brazil. The inclusion criteria were: a) minimal experience of 6 months with a RT program; b) minimal weekly frequency of three days; c) have not been exposed to any protocol of high intensity metabolic training with reduced rest intervals between sets. Exclusion criteria were: a) report the use of anabolic steroids; b) report any injury or medical limitation that prevented the execution of the RT protocols.

All the methodological procedures of the present study followed the rules of the National Health Council (466/2012), on researches involving humans. The subjects were integrated into the research only after signing the free and informed consent form stating their participation voluntarily in the study. The research was approved by the Ethics Committee of the Pontifical Catholic University of Paraná (PUC-PR), with this protocol: 38764314.0.0000.0020.

Procedures

Body composition

The body composition assessment consisted in the measurement of body mass (BM) and height (H) to determine body mass index (BMI). The BM measurement was performed on a weight balance (Toledo®, model 2096, São Paulo, Brazil) with an accuracy of 0.1 kg. The participant stood on the center of the weight balance, with her back to the scale in anatomical position, with the body mass distributed equally over both feet, and the

arms remaining loose along the trunk. The H, in centimeters, was measured in a Stadiometer (Sanny®) fixed to the wall (model Standard, São Bernardo do Campo, Brazil), staggered at 0.1 cm and defined as the corresponding distance between the plantar region and the formed vertex by the device cursor placed at the highest point of the head with the participant in inspiratory apnea. The BMI was calculated by the ratio of BM and H squared (BMI = kg/m²).

Maximal dynamic strength (1RM) test

Maximal dynamic strength was determined using a maximal repetition test (1RM), following the Baechle and Earle procedures. The test started with a specific joint warm-up composed of 3 sets of 10 repetitions with low-load. The determination of 1RM was measured for the Leg Press. The exercise was elected to quantify the 1RM as it is a basic exercise that carries a greater load and allows a more precise determination on the execution of the movement in this condition. It also enables a safer test to be performed where the researcher can also offer better technical support in the event of a failure or unexpected event. Participants were instructed to lift their weight only once. After the movement was completed, the load was increased and another attempt was made after 3 minutes of rest interval. The same procedure was repeated until the participant did not lift the load once with the appropriate technique. The last load used with the execution of the appropriate technique of movement was recorded as the value of 1RM.

Blood Lactate

A small blood sample (25µl) was taken from the right ear lobe just before and immediately after the subjects complete each RT protocol to determine blood lactate levels. Blood from these incision was allowed to flow through a heparinized (NH4) capillary tube. From the capillary tube, blood was added to a Eppendorff tube and filled with a 1:3 buffer solution (blood to buffer). These samples were then stored at approximately 4º Celsius for about 30 minutes and then placed in a refrigerator for another time to be analyzed by YSI 1500 lactate analyzer (Yellow Springs Instrument Co., Yellow Springs, OH).

Blood Analisys

Participants were fasted for 4 hours prior to pre-exercise blood collection, which was obtained from the forearm (10 mL) between 6- and 7-PM. Blood samples were collected again immediately after the end (IP) of the protocol, and repeated at 15-, 30- and 60-minutes after the end. No food or liquid was consumed during protocols. Blood samples were centrifuged at 2500 rpm for 15 minutes at 4º Celsius. Plasma samples were frozen and stored at -80º Celsius until analyzed.

Experimental Protocol

After performing the 1RM test and retest, all participants performed 2 randomized training protocols (DS or MS) separated by 7 days between each of them. Both sessions started with a warm-up that consisted of 15 repetitions with 50% of 1RM in the Leg Press. The interval between the end of the warm-up at the beginning of the protocols was 2 minutes. The protocol performed for MS were 3 sets of 10 repetitions at 75% of 1RM, with 1 minute of rest interval between sets. DS performed the same exercise by performing 3 sets consisting of: 10 repetitions at 80% of 1RM in each sets, followed by another 10 reps at 60% of 1RM and 10 reps at 40% of 1RM, with no rest interval. After completing this sequence with 3 load reductions, the rest interval was started, which was 45 seconds. The protocols were not intentionally equalized in order to avoid the decharacterization of the DS protocol, which aims to be intense and more volume lifted than MS. This conduct was adopted to increase the extrapolation of the data, since RT practitioners do not seek to equalize the training loads in their daily lives.

Statistical Analyses

The data were tabulated and stored in a database developed in the Microsoft Office Access 2003 program. All data were analyzed in the Statistical Package for Social Sciences (SPSS, version 18.0) for Windows, with a significance level stipulated in $p<0.05$ for all analyzes. First, normality of data distribution was confirmed by the Shapiro-Wilk test, and intraclass correlation (ICC) was sequentially used to test the homogeneity of 1RM loads. To present the characteristics of the participants, descriptive statics were used, with measures of central tendency and dispersion (mean and standard deviation). In the comparison between the means of the dependent variables (testosterone, lactate, monocytes and lymphocytes) for the different moments between the protocols (MS and DS), a two-way analysis of variance (ANOVA) was used, and *post-hoc* Bonferroni test to identify possible differences. The assumption of sphericity of the data was verified by means of Mauchly, followed by the Greenhouse-Gesseir correction if it was violated. The effect size calculation was performed to determine the magnitude of the differences. The scale proposed by Rhea (2004) was used to classify the magnitude of effect size (0-0,35 = trivial; 0,35-0,85 = small; 0,85-1,5 = moderate; > 1,5 = large). The sample size was obtained through an analysis in the G*Power 3.1 software using parameters for *F* family test

(ANOVA). The values adopted for the calculation were border power of 0.80 with α= 0.05 and an effect size of 0.34.

Results

The subjects characteristics are presented as mean (M) and standard deviation (±SD) in Table 1. The average results of the two days measurements for reproducibility of the 1RM test did not show a significant difference. Regarding the limits of agreement, it was observed that in 95% of the cases the random error was between 2.92 – 4.93kg with r= 0.985 e ICC = 0.984.

Table 1 – Subjects characteristics.

Variables	M±SD
Age	22,5 ± 3,2
Height (cm)	176.3 ± 6.7
Bodymass (kg)	75.4 ± 6.9
BMI (kg/m^2)	24.6± 1.6
Bodyfat (%)	18.9 ± 5
1RM (test 1)	302.2 ± 30.6
1RM (test 2)	305.1 ± 31.1

BMI: Body mass index; 1RM: maximal load lifted for the Leg Press. *CI: mean error confidence interval.

The lactate responses are shown in Figure 1. The lactate concentration was significantly higher ($t_{(1,1)}$=-5195; p= 0.001; confidence interval [CI] de 95%= -7.71;-2.97) for the DS protocol (14.1 ± 2.9) compared to MS (8.7 ± 1.6).

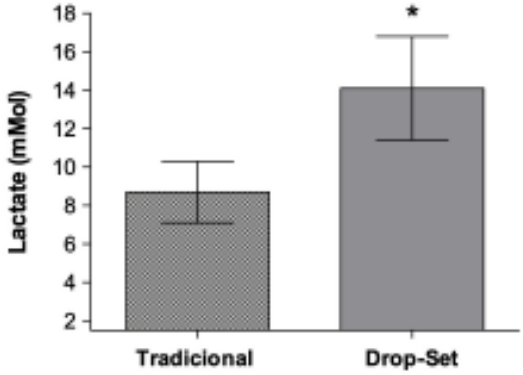

Fig. 1. Comparison of blood lactate concentrations immediately after (IP) the experimental protocols. *Significant difference between protocols (p<0.05).

Table 2 – Comparison of effect size for testosterone responses.

Groups	IP	Post 15'	Post 30'	Post 60'	Mean
Multiple-set (MS)	0.51 (small)	0.40 (small)	0.30 (trivial)	0.26 (trivial)	0.36 (small)
Drop-set (DS)	0.52 (small)	0.57 (small)	0.33 (trivial)	0.30 (trivial)	0.43 (small)

Rhea (2004) effect size scale: 0-0.35 = trivial; 0.35-0.85 = small; 0.85-1.5 = moderate; >1.5 = large. IP = immediately after.

The testosterone responses for the different protocols are presented in Figure 2. Testosterone levels increased significantly in both protocols at different moments. In MS, an increase in testosterone IP (304.5 ± 152.8) was observed in relation to baseline, post 30 minutes was higher than post 60 minutes (274.1 ± 121.1 and

233.8 ± 118.1, respectively). The DS presented higher IP values (380.7 ± 179.2) when compared to the baseline-, post 15 minutes (388.2 ± 177), post 30 minutes (345.6 ± 144.9) and post 60 minutes (299.7 ± 129.8). In the comparison between the groups, the DS showed higher values on the MS for the IP and post 15 minutes.

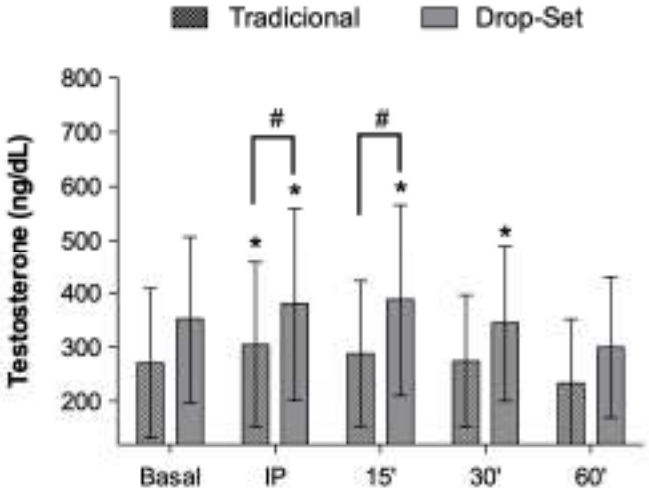

Fig. 2. Comparison of the responses of testosterone levels to the different protocols. *Significant difference between the different intragroup moments (p<0.05). #Significant difference between intergroup (p<0.05).

The monocytes responses to the different protocols are presented in Figure 3. The monocytes levels increased significantly in both protocols for the different moments. In the MS, monocytes showed an increase in the IP (545 ± 184.6) in comparison to the baseline (369.1 ± 112.1). Post 24 hours showed higher values in comparison to post 48 hours (420.7 ± 46.1 and 365.2 ± 78.5, respectively). DS had high IP values (571.4 ± 103.3) compared to baseline, post 24 hours (283.2 ± 112.1) and post 48 hours (365.2 ± 78.5). Among the groups, higher values were found for MS compared to DS in post 24- and 48- hours.

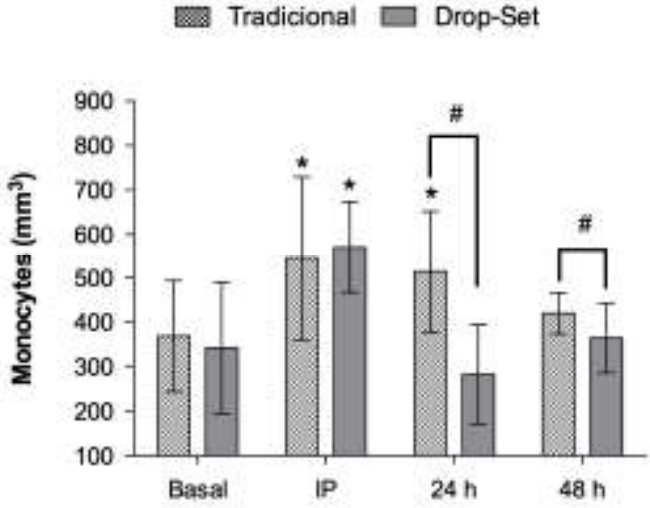

Fig. 3. Comparison of the responses of monocytes levels to the different protocols. *Significant difference between the different intragroup moments (p<0.05). #Significant difference between intergroup (p<0.05).

The lymphocytes responses for the different protocols are shown in Figure 4. The lymphocyte levels increased significantly in both protocols at different moments. In the MS, an increase of the lymphocytes at the IP (3779.5 ± 462.9) was observed in relation to the baseline (2955.5 ± 631.1), post 24 hours (2643.4 ± 601.1) and post 48 hours (2610.3 ± 536.1). In additionally, DS presented high values in the IP (4656.5 ± 904.5) when compared to baseline (3190.4 ± 787.3), post 24 hours (2797.7 ± 440.7) and post 48 hours (2797.6 ± 450.3).

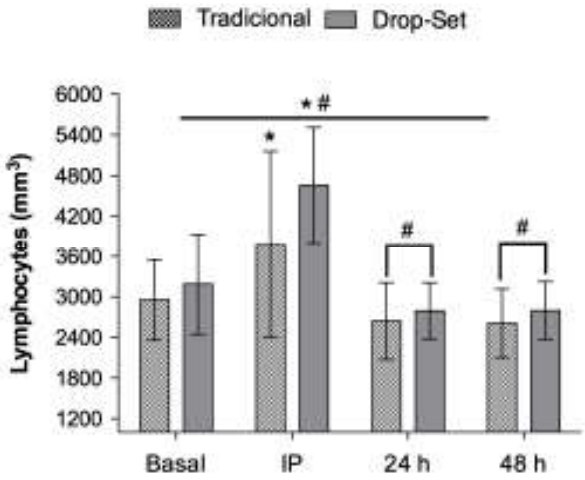

Fig. 4. Comparison of the responses of lymphocytes levels to the different protocols. *Significant difference between the different intragroup moments ($p<0.05$). #Significant difference between intergroup ($p<0.05$).

Discussion

The aim of the present study was to compare Multiple-Sets (MS) and Drop-Sets (DS) systems on acute testosterone responses and at different moments for lymphocytes and monocytes in recreationally trained men. It should be noted that the present study did not prioritize the equalization of the total volume of RT sessions, because our intention was to avoid the characterization of the protocol. In this case, DS aims to be intense and more voluminous than MS, that is, it is performed in this way by RT enthusiasts, who in turn, do not seek to equalize training in their daily lives. The observed results for lactate concentrations (MS: 8.7 ± 1.6 < DS: 14.1 ± 2.7) clearly demonstrated this condition (Figure 1), because DS promoted higher levels of work in relation to MS.

Although previous studies reports that voluminous RT sessions are efficient in increasing testosterone levels, the investigations compared only multiple-sets vs. a single-set (Gotshalk et al., 1997; Ratamess et al., 2005). In the present study, we used in the comparison with MS a protocol considered advanced that manipulates different variables during the execution of the sets. Therefore, our experimental design was elaborated in order to obtain a higher level of extrapolation of the data, since the great part of the studies prioritized the equalization of the total volume of RT session, not reproducing the reality of RT enthusiasts.

Our findings demonstrated that both protocols increased testosterone levels, however, DS presented higher values in contrast to MS for the IP and post 15 minutes (Figure 2). In this way, the results indicate that DS promotes an acute anabolic response greater than MS. The findings are in agreement with the literature considering that the investigations have already demonstrated that greater volume of training generates a higher testosterone response (Gotshalk et al., 1997; Mulligan et al., 1996; Smilios et al., 2003). However, this hormonal peak was observed only IP, lasting up to post 15 minutes after the end of RT sessions (Figure 2). But, previous studies report that this is enough to obtain a remodeling of the skeletal muscle (W. J. Kraemer & Ratamess, 2005; Smilios et al., 2003). The investigations suggest that in this short-term testosterone increase the levels of myofibrillar protein synthesis, leading to an increase in muscle mass and strength in both young and elderly. Part of these adjustments can be mediated by the growth factor-I system; insulin-like growth fator-1 (GH-IGF-1). When there is an elevation in testosterone levels, there is an increase in the concentration of IGF-1 in the muscle, optimizing protein resynthesis, relation between synthesis and breakdown (Crewther et al., 2008; W. J. Kraemer & Ratamess, 2005). Additionally, IGF-1 may also reduce rates of muscle protein breakdown, which could potentially influence the hypertrophic process (Ferrando et al., 2002). In this way, the evidence indicates that the muscular hypertrophy induced by the testosterone is associated to the increase of satellite- and myonuclear cells. However, the effect size showed that there was no difference in the magnitude of testosterone levels between the protocols at any moment. In addition, it was observed during the hormonal peaks (IP and post 15 minutes) for both MS and DS, only trivial values (Table 1) suggesting that even a larger volume of RT session is not able to potentiate testosterone secretion. Therefore, we can assume that the maximization of hypertrophic responses will not only occur through this pathway, because there is possibly a plateau in the elevation of testosterone secretion levels. Theoretically, this can be attributed to the amount of recruitment of motor units of strength and muscle mass. As the activation of muscle fiber increases, it allows better hormone-tissue interaction, that is, the more force imparted in a body segment with a large amount of muscle mass, the grater the testosterone response (Spiering et al., 2009). However, we know that both the increase in strength and the process of skeletal muscle

hypertrophy are progressive and gradual. Consequently, attempting to raise the magnitude of the acute levels of testosterone secretion simply by increasing the volume of the RT session becomes unlikely.

In contrast, the greater volume of training intensifies the mechanical stress, increasing the muscular damage. Microtraumas also stimulate growth factors that influence satellite cells in a regeneration pathway leading to the addition of myofibrillar protein. Our results demonstrated a significant increase in monocytes and lymphocytes for both protocols at different moments. The DS, as hypothesized, showed higher values at the IP-, 24 hours- and 48 hours- after compared to the MS, demonstrating that a greater volume of RT session generated a higher immune response. This indicates the occurrence of muscle damage after training.

Monocytes make up the second subpopulation of leukocytes to appear in the damaged tissue. When added it transforms into cells with phagocytic functions, approximately between 4 to 6 hours, now being called macrophages. One of its capabilities is to infiltrate tissues against chemotactic factors, adhesion, foreign particle phagocytosis and cytokine production. Therefore, the superior value of monocytes observed in the DS, suggest that the extent of muscle damage was higher compared to MS. Additionally, they did not surpass the reference values considered salutary. The lymphocytes showed an increase only at the IP moment and 24- and 48-hours after the training returned to the baseline levels. The values found for the different moments in DS were significantly higher than MS, and these levels indicated a lymphocytosis condition. This demonstrates that DS is capable of promoting more pronounced acute immune responses. However, 24 hours after the RT session, the immune responses were completely stabilized returning to baseline levels.

Conclusions

The present study provide evidence that DS promotes a significant acute increase in testosterone levels in relation to MS. However, the magnitude of this increase does not reflect large changes, suggesting that even RT sessions with more intensity and volume are no more efficient compared to traditional MS system in maximizing testosterone responses. The acute elevation of monocytes and lymphocytes was higher for DS indicating an inflammatory response and higher immunosuppression, but post 24 hours after the RT protocols, the values returned to baseline levels for both protocols. Thus, we can conclude that performing DS system does not cause deleterious effects related to muscle damage and immune system.

Conflicts of interest – The authors declare no conflict of interest.

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