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5 **LENGTH OF REST INTERVAL BETWEEN** 47
 6 **RESISTANCE EXERCISE SETS: PERFORMANCE** 48
 7 **AND INTER-INDIVIDUAL VARIABILITY OF CK ACTIVITY** 49
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33 **ABSTRACT** 75

34 **Purpose:** To determine how the rest interval (RI) lengths of 30 and 90 s between sets of biceps curl 76
 35 exercise affect the total volume of work performed and serum CK activity, and to verify the 77
 36 relationship between inter-individual variability of CK activity and total volume performed when the 78
 37 resistance exercise bout is conducted with 30 or 90 s **Methods:** Twenty-seven healthy sedentary men 79
 38 (18–20 years old) volunteered to participate in this study and were divided into two groups: 30RI 80
 39 ($n = 16$) or 90RI ($n = 11$), based on the RI length of 30 or 90 s between the sets of a resistance exercise 81
 40 protocol. The one repetition maximum (1RM) assessment of the elbow flexion was carried out and 82
 41 then a resistance exercise protocol which constituted five sets of biceps curl at 85% of 1RM with 30-s 83
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(30RI group) or 90-s (90RI group) RI length between sets was performed. Each bout was performed to voluntary fatigue and the number of repetitions and workout volume completed were calculated. Subjects provided blood samples prior to resistance exercise, and at 48, and 96 h following exercise to evaluate serum CK activity. The inter-individual serum CK activity along the 96 h after exercise was analyzed. **Results:** The results demonstrated that the longer RIs provided greater workout volume as expected, but there were no differences in serum CK activity between the groups. Additionally, it was possible to identify two high responder subjects, one from each RI group, who showed a great inter-individual serum CK activity variability. **Conclusion:** Exercising with short RIs does not appear to present any additional muscle microtrauma to untrained subjects. Further studies are necessary to evaluate if the inter-individual variability of the serum CK activity is influenced by the inter-set RI length.

Keywords: Recovery time; Exercise volume; Muscle damage; Muscular stress; Biochemical markers.

INTRODUCTION

When designing a program for resistance training, inter-set rest interval (RI) length is one of the variables used among others such as intensity, order of exercise, velocity of execution etc. The manipulation of the RI length variable is closely linked with volume workout and with the key objective proposed in resistance training programs. Despite many studies about this subject,^{19,35,38} the findings been largely unsubstantiated.⁶ Previous studies have examined the impact of inter-set RI lengths of 1 to 5 min between sets for single and multiple exercises.^{22,25,26,30,31,39–41} These studies demonstrated that, the shorter the RI length, the smaller will be the volume of work performed during a training session when it is done until volitional fatigue.

It is well-established that resistance exercise causes transient damage to muscle fibers.^{8,10} Quantifying post-exercise muscle damage provides vital information to the researchers and clinicians interested in evaluating resistance exercise protocols and the subsequent skeletal muscle response and adaptation. Assessment of the biomarker creatine kinase (CK) is widely used for indirectly evaluating muscle damage after resistance exercises in part because of its large expected increase from baseline and because it is

relatively simple and inexpensive to quantify.^{4,17,27} Interestingly, conflicting results appear in the literature when comparing the serum CK activity after resistance exercises when they are performed with different RI lengths. Mayhew *et al.*²⁴ evaluated the serum CK activity in men after ten sets of 10 repetitions of leg press [65% one repetition maximum (1RM)] using either a 1- or 3-min rest between sets. The group which executed the sets with a RI of 1 min had a higher CK elevation than the group who performed with longer intervals (3 min). However, Rodrigues *et al.*,³³ Machado *et al.*²¹ and Ribeiro *et al.*³² observed no differences in CK activity between research groups after completing a session of resistance exercise with different intervals lengths. Conflicting results can be attributed to great inter-individual variability on serum CK activity.⁷ For example, Machado and Willardson²² verify differences in serum CK activity following resistance exercise sessions with 1- or 3-min RI lengths when high responder (HR) subjects (i.e. subjects who had a great post-exercise serum CK activity peak) were separated from each group, while the analysis of groups without stratification (i.e. having both high and normal responders) demonstrated no differences between RI lengths.

Additionally, it is described that exercise with shorter than 1-min inter-set RIs length results in a

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1 significant increase in epinephrine, norepi-
 2 nephrine, cortisol, growth hormone and insulin-
 3 like growth factor-I.^{2,5,18,23} All these hormones play
 4 a role in the immunological response that occurs
 5 following heavy resistance exercises,²⁹ as demon-
 6 strated by concomitant elevations in prostaglandin
 7 E2 (PGE2), tumor necrosis factor- α (TNF- α),
 8 interleukin 1b (IL-1b), interleukin 6 (IL-6) and
 9 interferon- α (INF α),^{1,37} which may influence the
 10 muscle response to resistance exercises.^{1,18,19,37}

11 Based on previous findings we hypothesized
 12 that shorter inter-set RI length would manifest
 13 greater alterations in serum CK activity following
 14 an exercise session when compared to longer
 15 inter-set RI length. Therefore, the present study
 16 aims to determine how the RI lengths of 30 and
 17 90 s between sets of biceps curl exercise can affect
 18 the total volume of work performed and the
 19 serum CK activity. Additionally, we also aim to
 20 verify the relationship between inter-individual
 21 variability of CK activity and total volume per-
 22 formed when the exercise is conducted with RI
 23 length of 30 or 90 s.

25 METHODS

26 Subjects

27 The study consisted of twenty-seven healthy
 28 sedentary men aged between 18 and 20 years.
 29 They indicated that they were not currently using
 30 medical drugs, dietary supplements or anabolic
 31 steroids, and were without joint, muscular or
 32 cardiovascular diseases. The experimental con-
 33 ditions were conducted in accordance with the
 34 norms of the Brazilian National Health Council,
 35 under Resolution No. 196, promulgated on 10
 36 October 1996, referring to scientific research on
 37 human subjects and Helsinki Declaration (1964,
 38 reformulated in 1975, 1983, 1989 and 1996) of the
 39 World Medical Association ([http://www.wma.
 40 net/e/policy/17-c_e.html](http://www.wma.net/e/policy/17-c_e.html)), and all the subjects
 41 participated voluntarily.
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Table 1 Characteristics of Subjects.

| | 30RI (<i>n</i> = 16) | 90RI (<i>n</i> = 11) |
|----------------------|-----------------------|-----------------------|
| Age (years) | 18.5 ± 0.5 | 18.6 ± 0.5 |
| Height (cm) | 178.8 ± 7.1 | 178.6 ± 6.7 |
| Body Mass (Kg) | 68.3 ± 9.8 | 70.0 ± 14.5 |
| Biceps Curl 1RM (Kg) | 39.8 ± 6.4 | 38.8 ± 4.7 |

Design

Subjects were divided according to a computer
 generated randomization process into the 30RI
 (*n* = 16) or 90RI (*n* = 11) according to the RI
 length between sets of each group (i.e. 30 or 90 s).
 Comparisons of both groups in terms of age,
 height, body mass and dynamic strength were
 done prior to initial resistance exercise protocol
 and significant differences between groups were
 not found (*p* > 0.05). Descriptive characteristics
 of the subjects are showed in Table 1.

Methodology

The dynamic strength of the elbow flexor muscles
 was assessed through 1RM on a standard preacher
 curl bench on two different occasions separated by
 72 h. The same investigator measured 1RM for all
 subjects and verbally instructed subjects to per-
 form one full range of motion repetition, extend-
 ing the elbow to 170° and curling the weight back
 up to the shoulder with the weight at 100% of
 estimated maximum. If the lift was unsuccessful, a
 5-min rest was taken and the weight decreased
 slightly. If the lift was successful, a 5-min rest was
 taken and the weight increased. The procedure
 was repeated until subjects failed to complete a
 full range of motion lift. Weights were chosen so
 that the 1RM could be determined in three to five
 attempts. Maximum weight lifted was recorded in
 kilograms as the greatest amount of weight suc-
 cessfully lifted one time. The same investigator
 measured 1RM and provided verbal encourage-
 ment during each 1RM attempt for a given subject.

1 To increase the reliability of 1RM assessments, the
 2 following strategies were used: (a) the 1RM was
 3 measured on two nonconsecutive days that were
 4 separated by 72 h, (b) exercise technique was
 5 monitored and corrected as needed, and (c) all
 6 subjects received verbal encouragement. The
 7 highest 1RM load measured during the two
 8 sessions was used during the experiment. The
 9 1RM assessment procedures demonstrated high
 10 reliability (intraclass $r = 0.98$).

11 The exercise experimental protocol constituted
 12 of five sets of biceps curl at 85% of 1RM with a
 13 30-s (30RI group) or 90-s (90RI group) RI length
 14 between the sets. The subjects were instructed to
 15 extend the elbows from an elbow flexed (50° ,
 16 0.87 rad) to an extended position (170° , 2.97 rad)
 17 and then to return to the flexed position in 3 s
 18 (~ 1 s to concentric and ~ 2 s to eccentric phase).
 19 The repetition cadence for each exercise was
 20 controlled with a digital sound signal (Beat Test
 21 & Training, CEFISE, Nova Odessa, Brazil) that
 22 was adjusted so that each repetition was com-
 23 pleted in 3 s (~ 1 s concentric and ~ 2 s eccentric).
 24 Each bout was performed to concentric failure
 25 and the numbers of repetitions per set were
 26 recorded. The warm up consisted in two sets
 27 with 10 repetitions of same exercise with 30%
 28 1RM.

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Blood Samples Analyses

32 Blood samples were obtained from the subject
 33 while in a seated position from antecubital vein.
 34 Samples were placed into plain evacuated tubes.
 35 Samples were collected before exercise, after an
 36 8 h overnight fast prior to exercise bout, and at
 37 48, and 96 h following exercise. Immediately fol-
 38 lowing collection, blood samples were cen-
 39 trifuged at $1600 \times g$ for 20 min. The serum was
 40 removed and the serum CK activity was ana-
 41 lyzed with an enzymatic method at 37°C (CK-UV
 42 NAC-optimized; Biodiagnostica, Pinhais, Brazil)

in a Cobas Mira Plus analyzer (Roche, Basel,
 Switzerland). The CK assessment procedures
 demonstrated high reliability on quality control
 standards (intraclass $r = 0.97$).

Statistical Analysis

Data are presented as means [\pm Standard Devi-
 ation (SD)]. Comparisons of characteristics
 between groups were performed with Student's t
 test. To compare repetitions and volume, a 2
 (groups) by 5 (sets) Analysis of variance
 (ANOVA) were utilized. To compare serum CK
 activity, a 2 (groups) by 3 (CK) ANOVA were
 utilized. The alpha level was set at less than 0.05
 for a difference to be considered significant. Sig-
 nificant effects were further analyzed using pair
 wise comparisons with Tukey's *post hoc*s. The
 reliability of the CK activity assessments were
 assessed with the intraclass correlation (ICC) and
 the reliability was described as 'excellent' for ICC
 values in the range of 0.8–1.0 and 'good' for
 0.6–0.8, whereas values below 0.6 were con-
 sidered 'poor'.³⁶ The relationship between peak
 CK activity and total volume completed was
 performed using Pearson correlation. Statistical
 procedures were carried out with software
 package SPSS[®] (15.0) for Windows.

RESULTS

The number of repetitions per set and the volume
 for work performed is shown in Table 2. Number
 of repetitions and volume per set decreased sig-
 nificantly along the sets for both groups, but the
 30RI group displays a greater reduction when
 compared to 90RI group. Total number of rep-
 etitions ($p = 0.0001$), as well as total volume
 ($p = 0.0072$) was lower in 30RI when compared
 to 90RI.

The 2×3 ANOVA revealed no significant
 interaction between RI length and measurement
 time for CK activity ($p = 0.6040$). However, CK

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Table 2 Repetitions, Volume of Work Per Set and Their Total for Each Group.

| Set | Repetitions | | Volume (Kg) | |
|-------|----------------------------|--------------------------------|------------------------------|--------------------------------|
| | 30RI | 90RI | 30RI | 90RI |
| 1st | 9.1 ± 1.1 | 9.7 ± 0.7 | 237.1 ± 59.6 | 244.4 ± 30.9 |
| 2nd | 4.7 ± 1.1 ^a | 6.7 ± 1.6 ^{a,f} | 124.1 ± 46.7 ^a | 167.6 ± 35.2 ^{a,f} |
| 3rd | 3.1 ± 0.9 ^{a,b} | 4.7 ± 1.5 ^{a,b,f} | 82.3 ± 30.8 ^{a,b} | 119.0 ± 38.5 ^{a,b,f} |
| 4th | 1.4 ± 0.7 ^{a,b,c} | 3.5 ± 1.1 ^{a,b,d,f} | 38.5 ± 21.9 ^{a,b,c} | 88.0 ± 25.9 ^{a,b,f} |
| 5th | 1.1 ± 0.8 ^{a,b,c} | 2.3 ± 1.8 ^{a,b,c,e,f} | 27.7 ± 19.9 ^{a,b,c} | 61.1 ± 52.4 ^{a,b,c,f} |
| Total | 19.4 ± 3.4 | 26.9 ± 4.5 ^f | 509.7 ± 153.1 | 680.1 ± 141.4 ^f |

^aDifferent from the 1st Set for the same RI group ($p < 0.001$). ^bDifferent from the 2nd Set for the same RI group ($p < 0.001$). ^cDifferent from 3rd Set for the same RI group ($p < 0.001$). ^dDifferent from the 3rd Set for the same RI group ($p < 0.05$). ^eDifferent from the 4th Set for the same RI group ($p < 0.05$). ^fDifferences between RI groups on same Set ($p < 0.01$).

activity increased significantly at 48 h ($p < 0.001$ for RI30; $p < 0.032$ for RI90) when compared to pre-exercise measure in both groups (Table 3).

As expected all subjects reach a peak CK activity at 48 h after exercise protocol. No significant relationships were found between peak CK activity and total volume for 30RI ($r = 0.0053$) or 90RI ($r = 0.0151$). Neither of these correlation coefficients were significant ($p = 0.8454$ and $p = 0.9649$ respectively) (Fig. 1).

The volunteers showed a great serum CK activity inter-individual variability [Figs. 2(a) and 2(b)]. It is possible to identify two HR subjects, one from each group.

DISCUSSION

This study examined whether a standardized five-set elbow flexion/extension resistance exercise at

Table 3 Serum CK Activity at Different Times of Collection.

| | PRE | 48 h | 96 h |
|------|--------------|-------------------------------|----------------------------|
| 30RI | 189.7 ± 67.6 | 949.8 ± 1107.9 ^{a,b} | 506.4 ± 164.3 ^c |
| 90RI | 199.4 ± 70.6 | 743.8 ± 815.7 ^{a,b} | 403.8 ± 158.3 |

^aDifferent from PRE for the 30RI group ($p < 0.001$). ^bDifference from the PRE for the 90RI group ($p < 0.05$). ^cDifferent from 48h for the 30RI group ($p < 0.05$).

85% of 1RM could influence differently the performance and the serum CK activity response to the exercise carried out with different RI lengths. Besides, the inter-individual relationship between these variables was studied. Our main findings were that the performance, examined through number of repetitions and volume completed (i.e., load × sets × repetitions), was significantly reduced along the five sets of exercise protocol for both inter-set RI lengths, with greatest reduction for the shorter RI length. Additionally, the serum CK activity response after the exercise

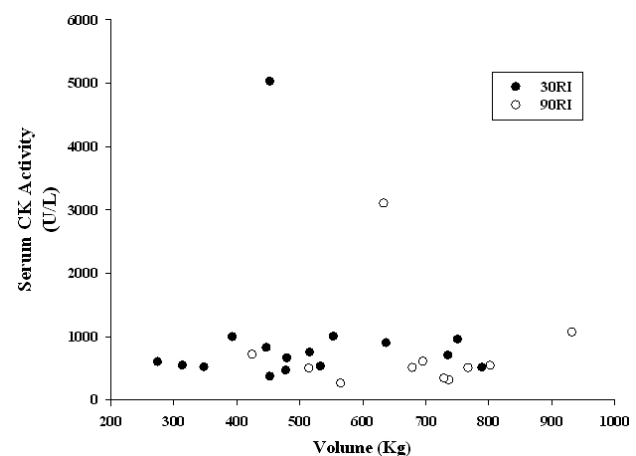


Fig. 1 Relationship between peak CK activity and total volume (sets × repetitions × load). Black = 30RI; White = 90RI.

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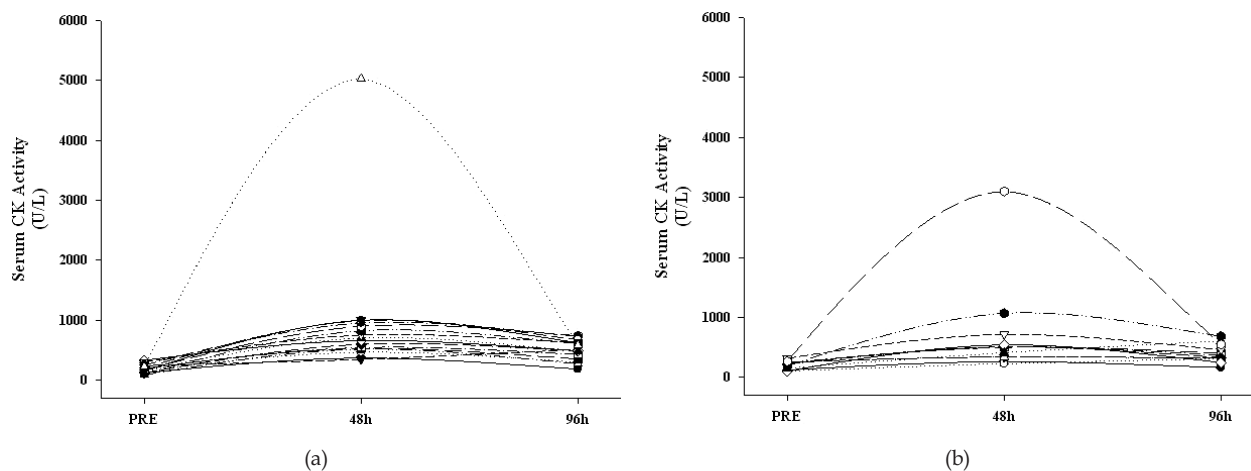


Fig. 2 Inter-individual variability of serum CK activity following resistance exercise. (a) 30RI subjects; (b) 90RI subjects.

was maintained significantly increased for a long time (i.e. at least 96 h after the exercise protocol) when the exercise was conducted with short inter-set RIs, but without differences between short (30RI) and long (90RI) inter-set RI lengths. The inter-individual relationship between performance and serum CK activity was not different for the 30RI and 90RI groups, demonstrating that these inter-set RIs induce similar muscle damage.

Many studies have examined the effect of inter-set RI length on performance of single and multiple exercises.^{11,21,24,32,33} Our findings about the performance were consistent with previous reports that compared the number of repetitions and the volume completed of a single exercise protocol, which is lower as when the inter-set interval length is shorter.^{39–41} Short inter-set RI lengths (i.e. lesser than 1 min) have been used in hypertrophy programs because it can induce a great metabolic stress, leading to an increased hormonal release, especially the release of the growth hormone.^{2,3,13}

Resistive exercise often triggers an increase in circulating levels of total CK via disruption of the sarcolemma. This disruption provides a pathway for the release of CK from the cytoplasm to the extracellular fluid, where it is transported to the

lymphatic system before entering the circulation. Total CK protein concentration in the circulation represents the balance between enzyme entry rate into the circulation versus clearance by the reticuloendothelial system.^{4,17,27} Serum CK activity can be elevated for 24–48 h following exercise bouts, with a gradual return to the basal levels in 72–96 h.^{9,34} The current study corroborates with previous investigations on this trend, where the serum CK activity was significantly elevated above pre-exercise levels at 48 h post-exercise. Our results represent a typical resistance response where the subjects tend to be submitted to a standard exercise, unlike from many studies where isokinetic exercises are used.^{7,28} Thus, the present results obtained with this design display findings which could be used by researchers, clinicians, coaches and fitness professional in day-to-day exercise prescription and evaluation.

The analysis of inter-individual variability of serum CK activity along 96 h after exercise demonstrated that two subjects, one from each RI group, had a great post exercise serum CK activity peak. According to Chen⁷ these subjects can be classified as HR, because of their CK induced by exercise variation. Recent studies have demonstrated that the variation of CK induced by exercise is great among the population and may

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1 depend on age, sex, and the fact that there are
2 subjects who present disproportional alteration of
3 serum CK activity.^{15,22} The evaluation of this
4 exercise-induced response is important because it
5 is proposed that HR subjects are more susceptible
6 to develop rhabdomyolysis, an undesirable con-
7 dition that occurs in response to strenuous
8 physical activity where the exercise induces stress
9 on the muscle causing damage to the muscle
10 fibers.^{9,14}

11 Another possible factor that has been attrib-
12 uted to responder status is the force application at
13 long muscle lengths. It has been shown that
14 subtle force applications at long muscle lengths
15 can alter a participant's high or low response to
16 the exercise.⁷ Because a force-angle curve was not
17 determined during muscle actions in this study, it
18 is possible that "responder" consistency was due
19 to the manner in which participants performed
20 the exercise, as well as the behavioral factors (i.e.
21 the speed of muscle actions during RI30 and RI90
22 or whether the participants had participated in
23 physical activity in the past) which may partially
24 account for the consistent response in this study.
25 We try to minimize these effects with control of
26 the cadence and including only subjects without
27 resistive training experience for at least 6 months
28 before the study.

29 It is interesting to note that the serum CK
30 activity in HRs before, and at 96 h after, exercise
31 protocol is similar to the other participants, cor-
32 roborating previous findings.²² There is no known
33 explanation for this fact, and so it is necessary to
34 develop further studies to elucidate whether the
35 highest serum CK activity after exercise is related
36 to the direct muscle damage or, a different kinetics
37 to wash out the CK from the serum.

38 We did not identify differences in serum CK
39 activity between groups which corroborates with
40 the findings of others.^{21,32,33} However, the pre-
41 sent result does not agree with the findings of
42 Mayhew *et al.*²⁴ In Mayhew's study the subjects

performed leg press exercises and the total work 43
had been equalized between short and long RI 44
conditions, whereas in this study each group 45
performed bicep curls to volitional fatigue for 46
each set (intervals modulate the number of rep- 47
etitions and the total volume) during the biceps 48
curl exercise. In addition, Saka *et al.*³⁴ demon- 49
strated that the lower body exercise results dis- 50
play lower CK activity variation when compared 51
with upper body, which can explain some of the 52
differences between our results and the results 53
from Mayhew *et al.*²⁴ Because of the different 54
architecture of arm and leg muscles,²⁰ it is prob- 55
able that mechanical stress per muscle unit differs 56
between these two muscle groups when doing 57
exercises of the same intensity. This can be one 58
of the reasons for different muscle damage 59
responses. In addition, Jamurtas *et al.*¹⁶ proposed 60
that submaximal eccentric actions during daily 61
activities, like downhill walking and going down 62
stairs, is a routine training stimulus for lower 63
body muscles. It is well documented that, fol- 64
lowing repeated bouts of eccentric exercise, the 65
muscles adapt themselves to protect against fur- 66
ther damage,⁹ lending support to the proposition 67
of other investigators.^{16,34} 68

69 Buresh *et al.*⁵ proposed that the differences
70 caused by exercise with short versus long RIs will
71 depend less on the RI employed and more on the
72 total volume (sets \times repetitions per set \times load) of
73 work completed. We tested this by examining the
74 correlation between the total volume
75 (sets \times repetitions per set \times load) and the peak of
76 serum CK activity. Our results showed a weak
77 correlation ($r = 0.0053$ and $r = 0.0151$, for 30 and
78 90s RIs, respectively) between cited variables.
79 With regard to endurance-based exercises, a re-
80 lationship is described between greater volume
81 and serum CK activity magnitude variations,⁴
82 but this data apparently is not repeated in
83 resistance exercises.¹¹ Friden *et al.*¹² demonstrated
84 that following eccentric exercises, myofibril

1 damage was more pronounced in type II muscle
2 fibers. Following a bout of eccentric exercise, type
3 II muscle fibers are more likely to be damaged
4 than type I muscle fibers and, therefore, show
5 greater serum CK activity. Resistance exercise
6 recruits more type II fibers than endurance exer-
7 cise; we postulate that CK response is less related
8 to volume and more related to intensity or other
9 physiological mechanism.

10 A mechanism of CK equal response in differ-
11 ent inter-set RIs can link with hormonal profile or
12 response. We did not examine a hormonal profile
13 and its influence on CK variability, but many
14 studies^{2,3,5,18,23} have shown augments in anabolic
15 hormones [growth hormone (GH), insulin-like
16 growth factor-1 (IGF-1) and testosterone] after
17 resistance training with very short inter-set RIs.
18 These augmentations are higher when the inter-
19 vals were shorter, and the higher effect of meta-
20 bolic membrane and protein degradation, and
21 higher mechanical load can be compensated by
22 acute augmented anabolic response. Despite little
23 empirical evidence about the influence of acute
24 rises in GH, IGF-1 and testosterone on muscle
25 hypertrophy,⁶ we do not neglect the short term
26 effect of these and other hormones on the main-
27 tenance of structural muscle architecture, but
28 further studies should be developed to evaluate
29 this topic.

30 CONCLUSION

31 The results from the current study add to the
32 growing body of knowledge regarding acute
33 responses to resistance training with different
34 inter-set RIs. Results showed that if sufficient time
35 is available, instituting longer RIs (e.g. 90 s) allows
36 for greater repetitions and total workout volume
37 compared to a shorter RI (e.g. 30 s). There might be
38 a point of diminishing returns at which the RI
39 between sets would become excessive, and yield
40 no further increases. This last point, however,

41 needs to be invested further. Differences in serum
42 CK activity modulation was not observed when
43 30- and 90-s inter-set RIs are compared.

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