Abstract:
The purpose of this study was to determine the effects of two resistance exercise (RE) protocols with different rest intervals between sets on oxidative stress and exercise-induced muscle damage. For this purpose, twenty untrained males voluntarily participated in the research and were randomly assigned to one of two resistance exercise groups: a) a shorter inter-set rest interval of 90 second (RI-90; n=10); or b) a longer rest interval of 180 second (RI-180; n=10). Resistance exercise in both groups consisted of chest press (CP), “lat” pull down (LP), leg extension (LE), leg curl (LC), and back squat (BS) exercises; these were done at a load of six repetitions maximum (6 RM). Blood samples were collected from the antecubital vein pre-exercise, immediately post-exercise, 6, 24, and 48 hours post-exercise, and analyzed for malondialdehyde (MDA) concentration and creatine kinase (CK) activity. The results indicated that both RI-90 and RI-180 caused significant changes in the MDA response (p=.003 and p=.036 in RI-90 and RI-180, respectively); MDA significantly increased six hours post-resistance exercise in both groups. Creatine kinase activity significantly increased at the 24-hour point post-exercise in both groups and continued for 48 hours post-exercise (p=.000 for RI-90 and RI-180). There was no significant difference between corresponding MDA and CK values of two groups. We conclude that the rest interval between sets of resistance exercise does not affect oxidative stress and myocellular damage.

Key words: weight training, oxidative stress, malondialdehyde, recovery, muscle cell damage

Introduction
Exercise of sufficient intensity and duration increases the formation of reactive oxygen and nitrogen species (RONS), creating an imbalance between oxidant and antioxidant levels. Such a condition, referred to as oxidative stress, can lead to the oxidation of endogenous lipids, proteins, and other molecules (Halliwell & Gutteridge, 1989). For example, it has been stated that resistance-based exercise (e.g. weight training) could lead to an increase in RONS in active muscle sites and increase lipid peroxidation (Güzel, Hazar, & Erbas, 2007). Thus, excessive resistance training may increase oxidative stress, promote cellular damage, and can cause redox imbalance in muscle cells (Liu, et al., 2005).

A widely supported hypothesis for the resistance exercise-initiated oxidative stress involves ischemia reperfusion injury and mechanical stress (McBride, Kraemer, Triplett-McBride, & Sebasti-anelli, 1998). Briefly, intense muscle contraction during resistance exercise (RE), particularly if isometric in nature, results in transient ischemia in the contracting muscle. With subsequent relaxation, reperfusion of the muscle produces rapid re-introduction of O₂ and abrupt formation of reactive species oxygen (ROS) (Viital, Newhouse, LaVoie, & Gottardo, 2004).

Some aspects of RE training, such as intensity, have been studied in relation to their effects on oxidative stress and antioxidant defense. For example, it has been reported that high intensity (high weight) RE induces free radical production more than a low intensity (low weight) RE program (Güzel, et al., 2007). However, there is little published data describing and examining the influence of different rest intervals between sets on plasma oxidative stress and redox state. The rest interval between sets has a critical role in RE prescription within a session, especially in the re-establishment of normal
blood flow following contraction. Anecdotally, the rest interval between sets may not be monitored as closely as other variables (e.g. intensity and volume) despite its known effects on metabolic, hormonal, and cardiovascular responses (Mavrommataki, Bogdanis, Kaloupsis, & Maridaki, 2006; Willardson, 2006). For example, a shorter rest interval between sets results in a greater hormonal stress response (Mavrommataki, et al., 2006; Kraemer, et al., 1990) and an increased serum CK activity (Mayhew, Thyfault, & Koch, 2005; Kafkas, 2014).

Functionally, different experimental designs show a decrease in performance when the rest interval is short, i.e. the fatigue is faster the smaller the (interval) time to rest (Richmond & Godard, 2004; Miranda, et al., 2007). On the other hand, where total load of training is controlled in strenuous resistance training, circulating oxidative stress markers are not different between a (slow) strength-based session and a (faster) hypertrophy training session. Based on these inconsistencies, there is a need for more research to determine the effect of rest interval on oxidative stress and serum CK activity. An improved knowledge of the effect of rest interval on these parameters may enable more effective training programs to be prescribed to both sports people and those undertaking RE for its well known health benefits.

Aside from the reperfusion hypothesis about oxidative damage following resistance training, it has also been hypothesized that RE leads to muscle cell membrane disruption as a consequence of both mechanical and metabolic stress. A muscle fiber, exhausted from strenuous work (concentric, but especially eccentric), exhibits increased membrane permeability associated with elevated cytoplasmic free calcium ions. This promotes the opening of membrane-based potassium channels, activation of proteolytic processes, and leakage of CK enzyme into the interstitial fluid and then plasma (Clarkson & Hubal, 2002; Brancaccio, Maffulli, & Limongelli, 2007). Soon after this, exercise-induced damage inflammatory cells infiltrate the damaged tissue and start to degrade cellular debris (damaged myofibrils, cytosolic components, and plasmalemma) through phagocytosis, production of proteases, and the release of reactive oxygen species (Tidball, 2005). Circulating CK activity has been used extensively as a marker for the extent of exercise induced skeletal muscle micro-injury (Brancaccio, et al., 2005; Mougios, 2007). It is reasonable to assume that post-RE circulating CK activity, would be higher if oxidative damage from exercise is greater. Thus, if rest interval between sets increases oxidative damage, CK would be expected to be also elevated.

The aim of this study, therefore, was to examine the effects of two different resistance exercise protocols performed at equal intensity in terms of rest interval between sets on the markers of oxidative stress and muscle damage in the plasma of healthy, untrained males. We hypothesized that a shorter (90-second) rest interval between sets would lead to greater post-exercise elevations in circulating lipid peroxidation markers and creatine kinase (CK) activity when compared to a longer (180-second) rest interval.

Methods

Participants

Twenty untrained men participated in the present study. Participants were randomized into one of two treatments: a shorter rest interval (RI-90) and a longer rest interval (RI-180) resistance exercise. All participants were healthy (there were no chronic or acute diseases), had no special nutritional habits, did not use medications or supplements, and were nonsmokers. The exclusion criteria were known cardiovascular and/or pulmonary disease, obesity, and hormonal abnormalities. No subject reported taking any exogenous anabolic-androgenic steroids, drugs, medication, or dietary supplements with the potential to affect redox and inflammatory responses to exercise during study. All participants read and signed an informed consent statement consistent with the University guidelines. The investigation was approved by the Committee on Use of Human Research Subject at Kurdistan University. Participants attended an information and familiarization session in which all details of research procedures were explained. The authors explained possible risks and discomforts associated with the exercise testing protocols for participants. Then participants were familiarized with the resistance training protocol that involved the upper body (UB) and lower body (LB) exercises. To gain familiarity with the equipment, practice proper technique, and avoid injury, participants completed one set of 15-20 repetitions with lightweight (3-5 kg) during the first session. During this period, free weight and training machines, used to perform chest press (CP), latissimus dorsi (“lat”) pull down (LP), leg extension (LE), leg curl (LC), and back squat (BS), were introduced to the participants, and they were informed about the correct technique of exercise performance.

Functional and physiological assessments

In their first day, between 9-10 a.m., a five ml venous blood sample was collected from antecubital region of the left arm in a seated position after 20 minutes of rest after a 12-hour fasting. Then, before any measurements, we asked the subject to remove excess clothing, overcoats and shoes, so that their body height and mass (Seca, Mod 220,
Germany) could be measured by an expert technician, and their body fat percentages estimated by means of measuring skin fold thickness (Lafayette, Mod 01127, USA) (Jackson & Pollock, 1985).

Then the 6 RM test was performed for BS, CP, LP, LE, and LC. To assess 6 RM, participants performed each exercise using a weight estimated to allow a maximum of six repetitions before muscular failure while using proper form. Resistance was adjusted on the basis of the participant’s ability to lift the initial resistance. If the participant performed more or fewer repetitions than six, the resistance was adjusted accordingly and the participant repeated the exercise. The 6 RM assessments were accomplished within one to three attempts allowing ample recovery time between sets. Before the initiation of the 6 RM test, a warm-up consisted of two sets with submaximal loads (16-20 rep) for each exercise was allowed. To increase the reliability of 6 RM testing, the 6 RM for each exercise was measured on two nonconsecutive days separated by 72 hours. To minimize error, the following strategies were adopted: standardized instructions concerning testing procedure were given to the participants before the test; the participants received standardized instruction about exercise technique; body position was held constant; and verbal encouragement was provided during the testing procedure (Simão, Farinati, Polito, Maior, & Fleck, 2005). The mass of all weights and bars used were determined using a precision scale. Physical characteristics of subjects at the start of the study are presented in Table 1.

Resistance exercise protocol

Two weeks after the familiarization period and physiological measurement session, a resistance exercise protocol for determination acute response of oxidative stress to different rest interval lengths was performed. Subjects participated in the resistance exercise at 10 a.m. after an overnight fast and following a prescription for minimal physical activity for seven days before this test.

Firstly, to warm up, participants performed one set of BS, CP, LP, LE, and LC exercises of 15-20 repetition each. After a warm-up and stretching exercises, both the RI-90 and RI-180 groups performed four 6 RM sets of each exercise with either 90 or 180 seconds of rest intervals between the sets. Participants completed one exercise and then the next exercise was started after a five-minute rest between exercises; this was identical for both groups.

In RI-90 group, verbal encouragement was given, especially during the 3rd and 4th sets to perform all sets to concentric failure and to complete a repetition. The participants were asked to perform this exercise as completely as possible. The same verbal encouragement was provided in the RI-180 condition. A spotter gave minimal assistance in the concentric phase if necessary so that six repetitions were completed on all four sets for each exercise. The total work by each group was calculated by the number of sets times the number of repetitions times the resistance load.

Blood collection, plasma preparation and biochemical analysis

After a 10-hour overnight fast and between 9-10 a.m. prior to the determination of physiological measurements and familiarization period, a five ml blood sample was obtained from the antecubital vein. For plasma collection, heparinized blood samples were centrifuged at 3000 (4°C) rpm for 15 minutes. The supernatants were separated from cells and transferred to sterile microtubes, and were stored at -70°C until analysis. Plasma samples were used for the measurement of malondialdehyde (MDA) and CK in duplicate and an average of the two samples was recorded. The same procedure took place immediately after, then 6, 24, and 48 hours after the completion of RE testing. MDA was measured based on the method of Buege and Aust (1978). Plasma CK activity, an estimate of exercise-induced muscle damage, was tested from blood samples using a Hitachi 912 biochemical device employing a standard kit (Pars Azmoon, Iran).

Statistical analyses

First, the Kolmogrov-Smirnov test was used to determine the normality of data distribution. Homogeneity of descriptive characteristics (body fat percentage and 1RM) of subjects at the beginning of the study was confirmed with the independent t-test. Intra-class correlation coefficients (ICCs) were used to determine 6 RM test-retest reliability. Dependent variables were compared using two-way ANOVA with repeated measures. Also, post hoc comparisons using the Bonferroni correction were applied to determine pairwise differences. All statistical analyses were carried out using the 17.0 version of SPSS. Values are expressed as M±SD. A p≤.05 level of significance was used.

Results

<table>
<thead>
<tr>
<th></th>
<th>RI-90</th>
<th>RI-180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>21.6±1.8</td>
<td>22.2±1.8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>71.8±3.6</td>
<td>73.1±4</td>
</tr>
<tr>
<td>Body height (m)</td>
<td>1.73±3.9</td>
<td>1.75±5.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24±6</td>
<td>23.1±1.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>21.6±2.3</td>
<td>19.8±3.4</td>
</tr>
<tr>
<td>1RM (chest press)</td>
<td>40.2±4.8</td>
<td>38.2±5.2</td>
</tr>
</tbody>
</table>
There was no significant statistical difference between RI-90 and RI-180 group in body mass index (BMI) and body fat percentage before and after the intervention. The 6 RM load intra-class coefficients for each exercise were as follows: CP=.91, LP=.92, LE=.96, LC=.93, and BS=.91. The results did not reveal a significant difference between RI-90 and RI-180 group in MDA and CK at baseline. Further, there was no significant difference between total work performed by RI-90 (4680±120 kg) and RI-180 (4251±78 kg) group after the exercise.

There was a significant main effect of RE on MDA (p=.032) and CK (p=.000) after resistance exercise. Results showed that there was a significant difference in MDA (p=.003) and CK (p=.000) after resistance exercise in RI-90 group. Post-hoc analysis showed that MDA increased significantly six hours after cessation of the exercise (p=.028) compared to the pretest levels, but then declined through to the pre exercise levels after 24 hours. Also, CK levels were significantly increased 24 h after the exercise in RI-90 group (p=.000) compared with the pretest. This increase continued 48 hours after the exercise (p=.000). Also, we found that there was a significant difference in MDA (p=.036) and CK (p=.000) after resistance exercise in RI-180 group. Post-hoc testing showed that MDA increased significantly six hours after cessation of the exercise (p=.040) compared to the pretest, but then declined through to the pre exercise levels 24 h after the exercise. Plasma CK activity was significantly increased 24 h after the exercise in RI-180 group (p=.000) compared to the pretest activity; this increase continued 48 hours after the exercise (p=.000). However, there was no significant exercise type x time interaction either for MDA (p=.93) or CK (p=.250) levels. This means that there was no significant difference between RI-90 and RI-180 group in MDA and CK at any time point after the exercise (see Table 2).

**Table 2. Changes in the levels of malondialdehyde (MDA) and creatine kinase (CK) before and after resistance exercise**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre (baseline values)</th>
<th>Immediately post</th>
<th>6 hr</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml) (RI-90)</td>
<td>1.2±.2</td>
<td>1.3±.3</td>
<td>1.9±0.4#</td>
<td>1.4±.2</td>
<td>1.3±.3</td>
</tr>
<tr>
<td>(RI-180)</td>
<td>1.3±.2</td>
<td>1.4±.6</td>
<td>1.9±0.5#</td>
<td>1.3±.2</td>
<td>1.4±.3</td>
</tr>
<tr>
<td>CK(U/l) (RI-90)</td>
<td>115±12.2</td>
<td>155.3±21.6</td>
<td>262.3±16.7</td>
<td>547.8±73.4#</td>
<td>460.2±62.1#</td>
</tr>
<tr>
<td>(RI-180)</td>
<td>102.5±12.4</td>
<td>142±17.2</td>
<td>272.8±8.1</td>
<td>506±78.6 #</td>
<td>435.5±82.4 #</td>
</tr>
</tbody>
</table>

The values are presented as M±SD of (RI-90) and (RI-180). MDA=malondialdehyde; CK=creatine kinase

# indicates significant differences in respect to baseline values p<.05

**Discussion and conclusions**

This study examined the effects of different inter-set rest intervals during a REtraining on plasma levels of MDA and a marker of exercise-induced muscle damage (plasma CK activity). Participants were exposed to exercise at either RI-90 or RI-180 rest conditions between sets of resistance exercise. Although we found that plasma CK activity increased significantly 24 hours after RE in both the RI-90 and RI-180 group, the changes of CK activity were not influenced by the rest intervals between sets. Further, we found that MDA was affected by both the RI-90 and RI-180 rest conditions following exercise, but the difference between the groups was not statistically significant. Plasma MDA concentration after RE in both groups increased significantly six hours after the exercise compared to the pretest, but then declined through to the pre exercise levels after 24 hours post exercise.

Our data reinforce the observations of Hudson et al. (2008) who showed that, when total training session load was controlled, a (quicker) hypertrophic training session produced the same level of oxidative stress as a (slower) strength training session; the latter including a longer rest interval between sets, albeit shorter, heavier sets. The present study takes these findings one-step further, isolating the effect of the rest interval whilst controlling for the actual lifts. Thus, the evidence now favors that rest interval between sets of resistance exercise do not impact total session oxidative stress.

Previous research has shown that exercise intensity may be the primary factor in the production of free radicals (McBride, et al., 1998; Güzel, et al., 2007). It has been reported that muscle acidity during intense exercise indicates a possible drop in the concentration of cytoplasmic nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, cofactors required by a number of free radical scavenging enzymes for activity (Lovlin, Cottle, Pyke, Kavanagh, & Belcastro, 1987). Muscle lactate concentrations would clearly be affected by a rest interval. In agreement with the current study, it has been reported that peak changes in MDA occur at around six hours post-exercise, while some studies have only examined immediate post-exercise MDA values (Maughan, et al., 1989). Some of these investigations, which have examined resistance type exercise and free radical formation, reported no increase in free radical formation (Sahlin, Cizinsky, Warholm, & Hoberg, 1989). Some of these investigations, which have examined resistance type exercise and free radical formation, reported no increase in free radical formation (Sahlin, Cizinsky, Warholm, & Hoberg, 1989).
In the current study, plasma CK activities were significantly elevated above pre-exercise levels 24 and 48 hours after the exercise. This demonstrates that there are no significant differences in the muscle damage processes which release intracellular CK into the interstitial tissue when rest interval is changed, at least from 90 to 180 seconds. In contrast, previous studies investigating different rest intervals between sets and exercises have elicited a differing response (Kraemer, et al., 1990; Mavrommataki, et al., 2006; Willardson, 2006). For example, Kafkas (2014) recently demonstrated that LDH concentrations were different after 1-minute rest versus 3-minute rests between concentric efforts at 60°/s and 120°/s. On the other hand, and in accordance with our findings, the group of Machado (Machado, et al., 2011) reported that the mechanical stress imposed by the four resistance exercise sessions invoked similar damage to muscle fibers independent of the rest interval (60, 90, 120, and 180 seconds) between sets. They concluded that the accumulated volume of work is the primary determinant of muscle damage in trained subjects who are accustomed to resistance exercise with short rest intervals. Actual CK responses to exercise are associated with factors such as aging, muscle fiber type, muscle group (upper or lower body exercise), and movement speed; all these factors can help explaining consistencies in the published studies (Koch, Pereira, & Machado, 2014).

Plasma CK, on the other hand, is argued by some not to be a particularly reliable marker of the extent of exercise-induced skeletal muscle damage (Barnes, Mundel, & Stannard, 2010), though it is very commonly used as such. A major limitation of the current study was that changes in strength were not measured as a result of training. Improving strength is the primary reason why many people do this type of exercise, and if changed rest interval affected strength gains, then any effect on oxidative stress seemed unimportant.

In conclusion, resistance exercise increases plasma MDA concentration and CK activity regardless of whether the inter-set rest interval was 90 seconds or three minutes. The extent of mechanical and oxidative damage is more likely a function of the total work performed and the intensity rather than the extent of rest between efforts. Nevertheless, more work is needed to broaden our understanding of the potential role of rest interval length between sets of RE on oxidative stress, especially since this form of anaerobic exercise is the one most widely prescribed as a component of a well-rounded fitness program. Many people may be considering the implementation of resistance training as a method to improve muscle strength and health. This study demonstrated that the rest interval between sets has little effect on muscle damage markers and oxidative stress when training with a 6 RM load.

Declaration
The authors have no conflict of interest to declare in the generation of this research and publication of this manuscript.

References


