EFFECTS OF VERY SHORT REST PERIODS ON IMMUNOGLOBULIN A AND CORTISOL RESPONSES TO RESISTANCE EXERCISE IN MEN

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ABSTRACT
There are few data relating to stress levels and immune function, prior to, and following acute bout of resistance exercise with varying rest intervals between sets in young resistance trained men. Therefore, the aim of this study was to determine the effect of varying rest intervals on serum IgA and cortisol concentrations during heavy resistance exercise (RE). Ten resistance-trained men completed 3 exercise bouts of 4 sets of bench press and squat to exhaustion at 85% of one repetition maximum (1RM) with 60-, 90- or 120-second rest intervals. Blood samples collected at rest (PRE), immediately post-exercise (POST), and 30-min post-exercise (30Post) were analyzed for IgA, cortisol and lactate levels. Data were analyzed using 3×3 repeated measures analysis of variance. The results showed there was not any significant differences between serum IgA levels of three protocols in pre-test and post-test (P>0.05). However, serum cortisol concentrations were significantly different between and within protocols (P < 0.05). We conclude that short rest intervals between sets in resistance training programs do not suppress IgA secretion. However, short rest intervals induce increase in serum cortisol concentration.

Key words: Serum immunoglobulin A, rest intervals between sets, cortisol


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INTRODUCTION

Immunoglobulin (Ig) is a general term describing a class of glycoprotein’s produced by mature B lymphocytes that appear in serum and secretions (e.g., saliva, tears) protecting mucosal surfaces. Ig exists in five classes (A, B, D, E, and G) with different functions. The exercise literature has focused mainly on IgA in mucosal secretions (e.g., saliva) and IgG in serum. Because serum and salivary IgA (sIgA) concentrations regulated independently, so their response should be different to exercise.

Regarding the sIgA concentration, previous studies have reported contrasting results, with sIgA concentration being acutely increased (Bishop et al., 2000; Blannin et al., 1998; Dimitriou et al., 2002), unaffected (Housh et al., 1991; McDowell et al., 1991) or decreased (McDowell et al., 1992; Tharp & Barnes, 1990; Tomasi et al., 1982) after exercise. However, there have been relatively few studies regarding the effect of exercise on serum IgA concentration. Acute exercise and moderate exercise training cause little, if any, change in the concentration of total serum Ig, Ig subclasses (e.g., IgG, IgA) or serum antibody titers to specific antigens (Mackinnon, 1996). In contrast, prolonged periods of intense exercise training may cause reduced serum Ig levels; for example, clinically low IgA and IgG levels were observed in resting samples obtained from elite swimmers (Gleeson et al., 1995). Green et al. (1981) reported that serum IgA concentrations were not different between fast runners and slower runners. In addition, Nehlson-Cannavella et al. (1991) demonstrated that there were no significant difference in serum Ig-A, -G, and -M after 15 week of moderate training in trained and control subjects.

Indeed, changes in serum IgA concentration following resistance exercise (RE) have not been study yet. Stress caused by RE can be manipulated by RE variables; originally defined by Kraemer (1992) including the number of training sets, number of repetitions, training intensity, training volume and rest between sets. Among these variables, rest interval between sets in RE has a special important, which is defined as the time between the ends of training set and commence of the next set so that body condition of the individual approached to the physiological stance before the activity. Previous studies have shown that rest interval length between sets affects the metabolism, cardiovascular function, hormonal response, also number of repetition in subsequent sets (Boroujerdi & Rahimi, 2008; Rahimi, 2005). RE with short rest intervals (1 min or less) leads to greater increment in catecholamines, cortisol, and growth hormone than RE bouts with long rest intervals (Raemer et al., 1996; Mayhew et al., 2005; Bottaro et al., 2009). These hormones all have been implicated as causative agents behind the immunological change that occur as a result of RE protocol. However, there is little information related to immune response to rest interval length between sets in RE.

Although the physiological mechanisms underlying the temporarily suppression various aspects of immune function after high-intensity exercise are still unclear, it is likely that both neural and endocrine factors influence the immune response to exercise (Fleshner, 2000; Pedersen & Steensberg, 2000). Cortisol is the end product of the neuroendocrine stress response in humans. Although high levels of cortisol have been demonstrated to inhibit antibody production in vitro (Ambroise, 1966), Fleshner (2000) suggested that elevated glucocorticoids are necessary, but not sufficient, to suppress the antibody response. However, while some studies have reported no acute changes in cortisol secretion in response to resistance exercise (Dohi et al., 2001; Nieman
et al., 1995), others have reported changes (Raemer et al., 1996; Och et al., 2001). Moreover, in relation to rest intervals between sets, previous (Kraemer et al., 1993; Ahtiainen et al., 2005) studies reported that short rest intervals are associated with an increase cortisol response after RE.

To our knowledge, there are few data relating to stress levels and immune function, prior to, and following acute bout of resistance exercise with varying rest intervals between sets in young resistance trained men. Therefore, the purpose of this study was to determine the effect of short rest periods on serum IgA and cortisol concentrations during heavy resistance exercise. We hypothesized that a shorter rest interval would yield greater decline in serum IgA concentration immediately post-exercise and during exercise recovery due to a greater rise in cortisol and other neuroendocrine factors in the circulation.

METHODS

Experimental Approach to the Problem

The acute IgA and cortisol responses of three resistance training protocols differing by rest periods between the sets (60, 90, and 120 second) were studied with 10 experienced resistance-trained college-age males. RE protocols were performed to failure and expected to lead to large acute hormonal responses. We hypothesized that when using short rest periods between the sets in resistance training to failure (maximum repetitions per sets); the immune and hormonal response should be larger along with a greater metabolic stress (i.e., lactic acid) than that of long rest periods between the sets.

Subjects

Ten experienced resistance-trained college-age males (age, 22 ± 2 years; weight, 84 ± 8 kg; height, 178.5±8.5 cm; at least 1 year of RE experience) volunteered for this study. Subjects were informed about the aims, nature and potential risks the study and provided written informed consent to take apart prior to the investigation. The study protocol was approved by the Ethics Committee of the Department of Sport Sciences, Azad University of Mahabad. Subjects were in a good health and on their ordinary diet, not permitted to use nutritional supplementation and did not consume anabolic steroids or any other anabolic agents known to increase performance.

Experimental design

The subjects were familiarized with the experimental testing procedures during a control day about one week before the actual measurements. Resistance load verification for the experimental bench press and half squat exercises were also determined. All of the subjects went through three strength exercise trials of different rest intervals between sets. The resistance exercises lasted from 09 00 hours to 11 00 hours and to avoid any potential carry-over effects and threats of internal validity, each of the three protocols was performed in a counterbalance order by all 10 participants. At least 48 h but not more than 72 h of recovery time was allowed between each training session. During the control day, three blood samples were obtained from each subject. One blood sample was drawn in the morning after 12 hours of fasting and approximately eight hours of sleep for determination of basal serum hormone and IgA concentration. Two blood samples were also drown within ½ hour without exercise at the same
time of day that each subject would later under take his heavy-resistance loading protocols of normal diurnal variation of serum hormone concentration.

During the exercise sessions, blood samples (5 ml) were drawn from an antecubital vein into 10-ml serum vacutainer tubes at pre-exercise (Pre), immediately post- (Post) and 30-minutes post (30Post) exercise for measurement of serum IgA, cortisol and blood lactate concentrations. The experimental design comprised three separate sessions of a RE protocol; subjects were assigned in a random order a rest interval of 60s (P60), 90s (P90) or 120s (P120) between sets. The RE session consisted of four sets of squats and bench press to failure using 85% of 1RM with 4-min recovery between the exercises.

**Strength testing**

Lower and upper body maximal strength was assessed by using one repetition maximum actions (1RM). Warm-up consisted of a set of five repetitions at the loads of 40-50% of the perceived maximum. In the half squat (1RM), the shoulders were in contact with a bar, and the starting knee angle was 90°. On command, the subject performed a concentric extension (as fast as possible) of the leg muscles starting from the flexed position to reach the full extension of 180° against the resistance. The trunk was kept as straight as possible. A security belt was used by all subjects. All of the tests were performed in a squatting apparatus in which the barbell was attached to both ends, with linear bearings on two vertical bars allowing only vertical movements. During the 1RM bench press test, the subject was instructed to perform from the starting position a purely concentric action maintaining the shoulders in a 90° abducted position to ensure consistency of the shoulder and elbow joints throughout the testing movement.

An attempt was considered successful when the movement was completed through a full range of motion without deviating from proper technique and form. Spotters were present to provide verbal encouragement and safety for the subjects. To ensure that all subjects were moving at approximately the same velocity for each repetition, each set was timed using a handheld stopwatch. The spotter called out a cadence for the eccentric and concentric phases of each repetition. The repetition velocity consisted of a 3-second eccentric phase followed by a 1-second concentric phase. During the next 3 testing sessions, 4 sets of the squat and bench press were performed with a 60-, 90-, or 120-second rest interval between sets. A counterbalance procedure was used to determine the order of the rest interval between sets for each testing session. Subjects were allowed to continue with their normal workouts throughout the duration of the study with the following exceptions: (a) subjects were instructed not to perform training 48 h before the testing session (b) subjects were instructed not to change their eating patterns during the study. The values for 1RM were 105.62±18 kg for bench press, and 160.31±19.71 kg for squat.

**Hormonal analysis**

Blood samples (5 ml) were drawn from an antecubital vein into 10-ml serum Vacutainer tubes and after approximately 45 min, serum tubes were centrifuged at 3000 rpm (5000 g) for 10 min at room temperature. Serum was separated from blood cells and stored at -20 °C until analyzed. Serum IgA concentrations were determined by nephelometric method using MININEPHTM HUMAN IgA kit (The Binding Site Ltd., Birmingham, UK) and cortisol concentrations were determined using enzyme immunoassay (RADIM SpA-Via del Mare, 125-00040 Pomezia (Roma) Italia). To eliminate interassay variance, all samples for a particular assay thawed once
and analyzed in the same assay run. All samples were run in duplicate with a mean inter- and intra-assay coefficients of variances were 6.9% and 6.2% for serum cortisol and intra-batch and inter-batch precision were 2.60 % and 3.62 % for IgA. Lactate in plasma was analyzed enzymatic method (PARS AZMUN KIT). The CV's for lactate was <5%. All samples from each subject were analyzed on the same day.

**Statistical analyses**

All data are presented as mean ± SD. Serum IgA and cortisol concentrations were compared between treatments using a 3×3 (condition × time) repeated measures analysis of variance (ANOVA). Blood lactate concentration was compared using a 3×2 (condition × time) repeated measures ANOVA. In the event of a significant F ratio, Scheffe post hoc tests were used for pairwise comparisons. A criterion $\alpha$ level of $P \leq 0.05$ was used to determine statistical significance.

**RESULTS**

The volume of exercise per set for squat and bench press are shown in Figures 1 and 2, respectively. Training volume performed during the four sets of squat and bench press decreased over sets during three protocols. Total work performed comparison between groups (P30, P60, and P120) revealed no significant differences (P>0.05) in P60 (3603.81±816.05 kg), P90 (4175.50±939.89 kg) and P120 (4352.06±996.87 kg).

![Figure 1. IgA levels (mean ± SD) during pre-exercise, immediately post- and 30-min post exercise with the 60 (P60), 90(P90) and 120s (P120) protocols.](image-url)
Figure 2. Cortisol levels (mean ± SD) during pre-exercise, immediately post- and 30-min post exercise with the 60 (P60), 90(P90) and 120 s (P120) protocols.

* Significant difference with pre-exercise
† Significant difference with P120

No significant time effects and treatment × time interactions were observed for serum IgA concentrations (p>0.05). However, serum IgA concentrations increased from pre to post exercise by 15, 14, and 14 % in three protocols (P60, P90 and P120 respectively) but these changes were not statistically significant. Serum IgA concentrations then decreased to 1.97, 4 and 7.74% after 30 min post exercise but were still higher than the pre exercise levels (P>0.05) (Figure 1). Significant time effects and treatment × time interactions were observed for serum cortisol concentrations (p<0.05). Serum CO levels significantly increased immediately after and 30 min after in P60 and P90 from 133±18 up to 225±45 ng/ml and 152±39 up to 248±51 ng/ml, (respectively) (P<0.05). Post hoc analyses indicated significantly greater PRE-POST elevation for cortisol in the 60- and 90-second rest intervals when compared to the 120-second rest interval (p<0.05) (Figure 2). Significant time effects were observed for blood lactate concentrations but no significant treatment × time interactions were observed (Figure 3).
DISCUSSION

The present study examined the effect of a bout of resistance exercise with varying rest intervals between sets on serum IgA and cortisol concentrations of young resistance trained men. Immunoglobulin’s (Ig) are a part of immune system components, which are produced by the lymphocyte B in blood serum and tissue liquids in primates. An Ig that reacts with a specific antigen (foreign protein) is termed an antibody. An antibody has several functions; most importantly, the binding of antigen on the surface of foreign cells, which in turn stimulates other immune cells to kill the foreign pathogen. Acute RE has been reported to increase serum cortisol and epinephrine (Nieman et al., 1995; Kraemer et al., 1996), which are potential modulators of the immune system. Therefore, we hypothesized that acute RE with short rest intervals would result in a downregulation of immune function in resistance trained men. The main finding of the study were that serum IgA concentration were unaffected at post- and 30 min- post RE with short rest intervals of 60, 90, and 120 second but cortisol concentrations were significantly elevated following RE protocols in young resistance trained men. This findings support the results of several previous studies that demonstrated no change in IgA concentration following acute exercise (McDowell et al., 1991; Nehlsen-Cannarella et al., 2000). Also, these findings are in line with Walsh et al. (2002) reported that secretion rate of IgA was unaffected by the high-intensity intermittent exercise. The hypothesis that heavy RE would temporarily suppresses immune function as assessed by serum IgA levels immediately and 30-min post resistance exercise was not supported as well as short rest intervals between RE did not.
Although our study reported the 15, 14 and 14 % increase in serum IgA concentrations immediately after RE protocols (P60, P90 and P120, respectively), these were not significant (p>0.05). These findings indicate that four sets of bench press and squat exercise at 85% of 1RM with short rest intervals between sets do not suppress immune function in young resistance trained men. These findings are in line with study that reported three sets of eight repetitions at 70% of 1RM (Flynn et al., 1999) did not result in post-exercise suppression of immune function. In addition, the results are in line with those studies, which found no change in saliva IgA concentration after exercise (Housh et al., 1991; McDowell et al., 1991) several studies (Nieman et al., 2002; Novas et al., 2003; Mackinnon & Hooper, 1994) have reported falls in IgA concentration following intense exercise. Steerenberg et al. (1997) demonstrated that sIgA concentration did not change in 42 triathletes following an Olympic-distance triathlon race event in the Netherlands. The majority of the research indicates that the combination of high-intensity and prolonged-duration exercise is necessary before significant decreases in these immune parameters are detected (Mackinnon, 1996). Although our resistance exercise protocol was exhausting, it did not result in a decrease in serum IgA, which normally occurs after intense endurance exercise (Nieman et al., 2002; Novas et al., 2003; Mackinnon & Hooper, 1994). These discrepant findings may related to several factors, including nutritional and training status of the subjects, circadian rhythms, and the exercise protocol employed (Engels et al., 2003). The present study is the first to provide evidence of the effect of RE on serum IgA concentration and demonstrated that short rest intervals between sets do not affect on serum IgA concentration.

Although the physiological mechanisms underlying the change in IgA are still unclear, it is likely that both neural and endocrine factors influence the immune response to exercise (Fleschner, 2000; Pedersen & Steensberg, 2000). Regarding the endocrine factors, Ambrose (1966) demonstrated that high levels of cortisol could inhibit antibody production in vitro, Fleschner (2000) suggested that elevated glucocorticoids are necessary, but not sufficient, to suppress the antibody response. In terms of CO response to recovery between resistance exercise sets, Nieman et al. (1995) reported no significant increase in CO after exhaustive squat exercise in young men and a significant decrease in CO at the 2-h postexercise time point. These researchers allowed 180-second rest between sets, and Kraemer et al. (1996) previously reported that CO was significantly increased when 60-second, but not when 180-second, recovery was allowed. Our data indicated that serum CO concentrations were significantly higher in RE with 60- and 90-second rest between sets than 120-second rest protocol. However, we did not observe correlations between serum IgA and cortisol at any time of the trial, which is consistent with reports of a lack of relationship between levels of serum IgA and cortisol following high-intensity exercise (Tharp & Barnes, 1990) or 10 weeks of running training (McDowell et al., 1992).

In addition, the stimulation of β-adrenoreceptors increased IgA secretion in a dose independent manner above a certain threshold; however, prolonged β-adrenergic stimulation appeared to reduce the replenishment of IgA into the glandular pool (Proctor et al., 2003). Ring et al. (2000) suggested that the acute decrease in sIgA secretion rate during exercise was mediated by α1-adrenergic mechanisms. Therefore, the previous inconsistencies in responses of IgA secretion rate to exercise (Walsh et al., 2002; Bishop et al., 1999; Li & Gleeson, 2005) may be attributable to the interaction between different types of stimulation and their receptors during exercise. For example, when the α1-adrenergic stimulation is stronger than other types, such as β-adrenergic
activity, and is above a certain threshold, IgA output may be decreased. Conversely, when the β-adrenergic stimulation is stronger than α1-adrenergic stimulation, sIgA output increases.

The results presented here contribute to existing research in relation to the measured hormonal and immune function responses to heavy RE with short rest intervals between sets in young resistance trained men. Therefore, it is recommended that future studies consider heavy RE with short and long rest intervals between sets as an effective factor on hormonal and immune function responses. Further investigations are necessary to determine if these findings are generalizable to other populations.

CONCLUSIONS
In summary the result of the present study, demonstrate that heavy RE did not suppress IgA secretion, which is a part of immune system components, in young resistance trained athletes. In addition, different short rest intervals between resistance exercise sets do not induce reduction in serum IgA concentration. Whether alterations in circulating immunoglobulins are related to an increased risk of illness in athletes is unknown. However, it is premature to make any definitive recommendations at this point. Further research into the effects of resistance exercise on immune function and illness is needed. Additionally, an acute bout of heavy RE increases the extent of postexercise cortisol, which is dependent to rest periods between RE sets. The greater cortisol concentrations in shorter rest intervals between RE sets in this study confirm that acute bout of heavy RE produces significant physiological hormonal responses in young resistance trained men, but dose not decline serum IgA concentrations, which has important role in immunity.

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