



ORIGINAL ARTICLE

# Resistance exercise on two consecutive days induces cortisol, CK, IgA responses in active young males

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## KEYWORDS

Circuit resistance training;  
Catabolic hormone;  
Creatine kinase;  
Immune response

## Abstract

**Background:** Reduction in recovery time may prevent physiological variables from returning to pre exercise levels; therefore, it is likely that the athletes will have a decrease in immune response and experience increased stress. The purpose of the current study was to examine whether two consecutive or non-consecutive days of circuit resistance exercise (CRE) on cortisol, creatine kinase (CK) and immunoglobulin A (IgA) responses in active young men.

**Methods:** Ten healthy male university students ( $22.25 \pm 1.61$  years) performed two consecutive days of circuit resistance exercise (TCD-CRE) and two non-consecutive days of circuit resistance exercise (TNCD-CRE). Participants performed CRE at 75% of their one-repetition maximum (1RM). Blood and saliva samples were taken during baseline and immediately after exercise and analyzed for serum CK activity and salivary concentrations of cortisol and IgA.

**Results:** Based on the results, there were significant increases in cortisol and CK at post as compared with pre in the TCD-CRE group ( $P < 0.001$ ,  $P = 0.001$ ). Also, a significant increase in cortisol at post as compared with pre in the TNCD-CRE group was observed ( $P < 0.001$ ). Additionally, the level of IgA was significantly reduced post exercise when compared to pre value in the TCD-CRE group ( $P = 0.011$ ). On the contrary, there were no significant changes in concentrations of IgA and CK activity in the TNCD-CRE group ( $P = 0.11$ ,  $P = 0.24$ ). Moreover, there were statistically significant differences in cortisol and CK between the groups ( $P = 0.001$ ,  $P = 0.002$ ).

**Conclusion:** Based on these data, TCD-CRE causes more immunological responses, and thus may lead to trauma. It seems that there is a need for a 48-h recovery between exercise sessions to prevent the decrease in immune function by CRE.

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**PALABRAS CLAVE**

Entrenamiento de resistencia a los circuitos;  
Hormona catabólica;  
Creatina cinasa;  
Respuesta inmunitaria

## Ejercicio de resistencia en dos días consecutivos induce respuestas a cortisol, CK, IgA en hombres jóvenes activos

**Resumen**

**Antecedentes:** La reducción del tiempo de recuperación puede impedir que las variables fisiológicas vuelvan a los niveles anteriores al ejercicio; por lo tanto, es probable que los atletas tengan una disminución en la respuesta inmunológica y experimenten mayor estrés. El propósito del presente estudio fue examinar si dos días consecutivos o no consecutivos de ejercicio de resistencia de circuito (CRE) en las respuestas de cortisol, creatina cinasa (CK) e inmunoglobulina A (IgA) en hombres jóvenes activos.

**Métodos:** Diez estudiantes universitarios varones sanos ( $22,25 \pm 1,61$  años) realizaron dos días consecutivos de ejercicios de resistencia de circuito (TCD-CRE) y dos días no consecutivos de ejercicios de resistencia de circuito (TNCD-CRE). Los participantes realizaron la CRE al 75% de su máximo de una repetición (1RM). Se tomaron muestras de sangre y saliva durante el estudio inicial e inmediatamente después del ejercicio y se analizaron para determinar la actividad de la CK en suero y las concentraciones salivales de cortisol e IgA.

**Resultados:** En base a los resultados, hubo aumentos significativos en el cortisol y la CK en el post con respecto a los anteriores en el grupo de ETCRE ( $P < 0.001$ ,  $P = 0.001$ ). Además, se observó un aumento significativo del cortisol en el período posterior al tratamiento en comparación con el período anterior en el grupo TNCD-CRE ( $P < 0.001$ ). Además, el nivel de IgA se redujo significativamente después del ejercicio en comparación con el valor previo en el grupo TCD-CRE ( $P = 0,011$ ). Por el contrario, no hubo cambios significativos en las concentraciones de actividad de IgA y CK en el grupo TNCD-CRE ( $P = 0.11$ ,  $P = 0.24$ ). Además, hubo diferencias estadísticamente significativas de cortisol y CK entre los grupos ( $P = 0,001$ ;  $P = 0,002$ ).

**Conclusión:** Basado en estos datos, el TCD-CRE causa más respuestas inmunológicas, y por lo tanto puede llevar a un trauma. Parece que hay una necesidad de una recuperación de 48 horas entre sesiones de ejercicio para prevenir la disminución de la función inmunológica por parte de la CRE.

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**Introduction**

Nowadays, professional athletes and beginners are forced to spend many hours of exercise on a daily basis to improve their conditions, increase the number of daily practice sessions, or exercise on consecutive days due to extensive sporting competitions and lack of time. This reduction in recovery time may prevent physiological variables from returning to pre exercise levels; therefore, it is likely that the athletes will have a decrease in immune response and experience increased stress.<sup>1,2</sup> Studies showed that heavy exercise schedules in consecutive competitions have weakened athlete's immune system and gradually put them at risk for a variety of diseases associated with the immune system, including infections (upper respiratory tract infection (URTI) and pneumonia).<sup>1,2</sup> Many believe that the more intense the exercise is, the athlete body's resistance to diseases will increase and they will be healthier. This belief is rooted in the desirable effects of exercise on some body organs, including the cardiovascular and respiratory system; however, studies on repeated or prolonged exercises on consecutive days on the immune system showed the prevalence of URTI and degradation of immune factors.<sup>3</sup> Depending on the type, intensity, duration of exercise, and the recovery,

immune suppression can occur in athletes and may increase the likelihood of URTI.<sup>4</sup>

Research has shown that there was a significant relationship between hormonal and immune systems and exercise can have a direct or indirect impact on the performance of these systems. Activities and exercise training may reduce or increase the level of some hormones relative to rest. However, among these hormones, cortisol plays a significant role.<sup>1</sup> Cortisol is one of the possible causes of URTI in athletes due to immune suppression after severe or prolonged resistance or aerobic exercise.<sup>2</sup> In biological functions, cortisol reduces the number of neutrophils, eosinophils, lymphocytes and weakens the function of natural killer (NK) and T cells.<sup>5</sup> Research has reported a linear relationship between cortisol levels and intensity and duration of physical activity. Although cortisol has been shown to increase during exercise, its most significant changes are after exercise.<sup>6</sup> The effects of changes in some hormones, such as cortisol, during resistance (moderate to submaximal intensity) and endurance exercises on white blood cell count have been compared in some studies.<sup>7</sup> The results indicated that there are other factors other than cortisol that may affect the changes in white blood cells.<sup>1,2</sup>

Increased serum cortisol has a significant correlation with muscle damage markers 24 h after exercise.<sup>8</sup> Creatine kinase (CK) is one of several markers (lactate dehydrogenase (LDH), myoglobin, troponin, etc.) of muscle damage caused by exercise.<sup>9</sup> In general, studies have shown serum CK activity after exercise, which is poorly related to functional criteria for muscle pain, strength, range of motion.<sup>10</sup> Often, low CK increase after exercise is equivalent to less injury in sports, but not always.<sup>11</sup> In the general population, the serum CK levels varies between 35–175 U/L with a range of 20 to 16,000 U/L, and this is a widespread range indication of indirect occurrence of minor disorders and injuries, genetic factors, and physical activity status.<sup>12</sup> Several studies have shown changes in the activity of CK after exercise, and argued that these changes vary according to exercise conditions. For example, in isometric muscle contraction exercises, the peak in serum CK activity is observed relatively early and 24–48 h after exercise,<sup>13</sup> while peak CK activity has been observed 3–7 days after eccentric muscle contraction exercises.<sup>14</sup> There is a two-phase pattern in weight training. Totsuka et al. reported that the CK response depends on the level of individual fitness and is different between athletes and non-athletes.<sup>15</sup>

In addition to cortisol, immunoglobulins (Ig) also play an essential role in the immune system. Researchers have shown that exercise can change the amount of Ig. Based on scientific evidence, increasing cortisol concentration also has an effect on B lymphocytes during high-intensity exercise and reduces Ig.<sup>2</sup> Ig in the immune system plays an important role in protecting the body against infectious diseases. Following some stimuli, including acute exercise, B cells proliferate and are able to secrete and produce antibodies or Ig specific to the antigen that starts the immune response. The highest Ig circulation were reported in the IgG (about 12 g/L), followed by IgA (about 1.8 g/L) and IgM (about 1 g/L).<sup>16</sup> Most IgA secretions in the mucous membrane are used in the first line of defense against viral infections. When athletes tolerate a lot of pressure, changes in levels of Ig and their hormones occur.<sup>16</sup>

Many studies have examined the impact of aerobic exercise. Different studies have shown that endurance training with a moderate intensity has positive effects on the immune system and protect the body from infections; however, high-intensity endurance exercises have reduced the immune function in humans. In contrast, immune responses to resistance training with different intensities have not been well studied and little research has been done in this area.<sup>1,7</sup> Compared with endurance training, resistance training, in spite of needing less oxygen, can cause muscle damage and specific hormonal changes that affect the immune system.<sup>7</sup> Regarding the results of studies on the effect of exercises on the immune system, it seems that the intensity, duration and type of activity determine the changes rate in the immune system.<sup>1,2</sup>

Based on the practice guidelines, two to three days exercise per week with an interval of 48–72 h is appropriate for optimal muscle growth and muscle strength improvement.<sup>17</sup> However, so far, there has been little research on the impact of short-term recovery between exercise sessions. Also, there is no specific study on the effects of different recovery periods between circuit resistance exercise (CRE) sessions on the immune system and there is little information about

it. CRE seems to cause different changes in muscle injury indices and exercise stress due to the use of the main muscles and the high training pressure (75–85% 1RM). These factors can have different effects on the immune system, and it seems that recovery between exercise sessions is one of the key factors in controlling and modulating these training pressures on individuals. According to the stated contents, this study attempted to investigate the effect of 2 days of consecutive and non-consecutive CRE on the CK, cortisol and salivary IgA in active young men.

## Methods

Ten healthy male university students ( $22.25 \pm 1.61$  years) volunteered to participate in the study. The participants had been involved in resistance training for at least 6 months ( $10 \pm 3$  months). No participant had a previous history of cardiopulmonary disease or was taking medications during the study. Participants underwent a physical examination prior to enrolling in the study and they had no history of respiratory disease, spinal deformity and musculoskeletal disorders, endocrine or other disorders that would contraindicate participation in a heavy resistance training programmed. None of the participants had used any dietary supplements or medications for at least 12 months prior to this investigation. The researchers described the research process and procedures to the participants and asked them to fill out the consent form and general health questionnaire. The study was conducted in accordance with the Institutional Ethics Review Committee from the University and according to the Declaration of Helsinki

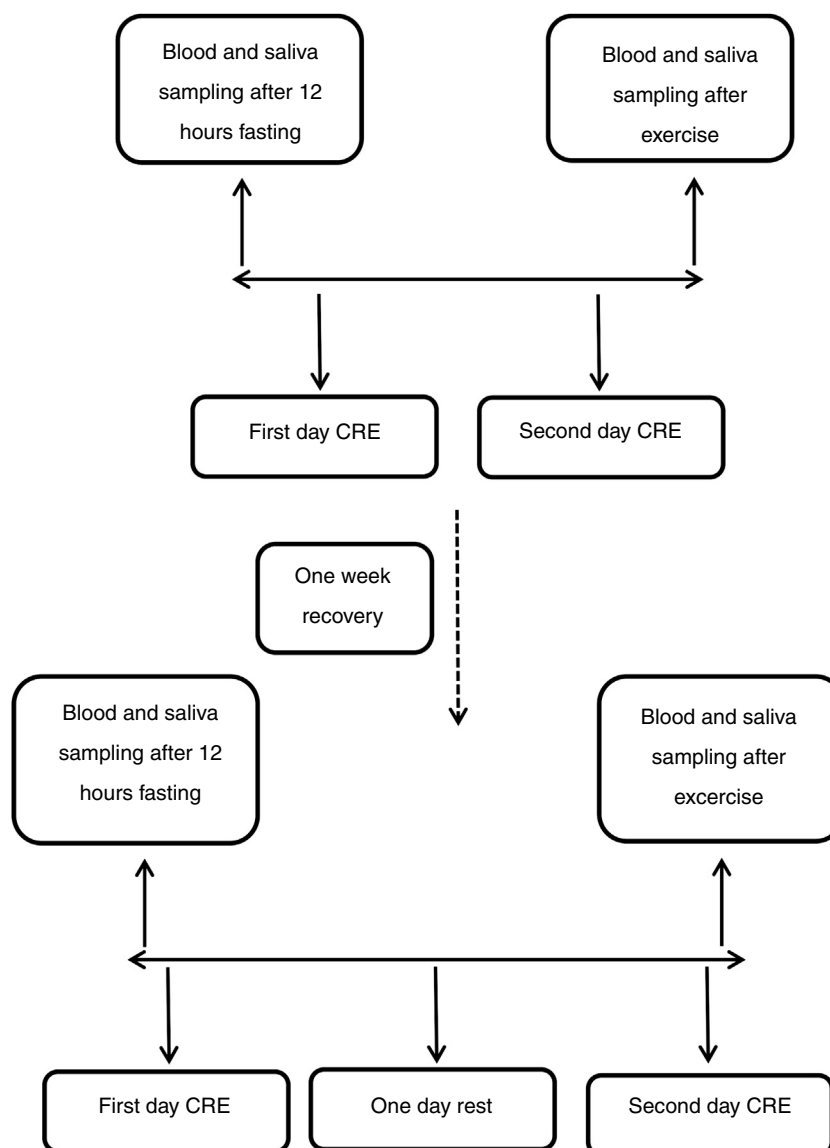
After explaining the procedures and working methods, measurements were carried out. Test to measure the strength of one repetition maximum (1RM) in the bench press, lat pull down, triceps curls, biceps curls, overhead press, vertical row, leg press, leg extension, push up, and sit up exercises were used. One repetition maximum, the maximum amount of weight that subjects can move it once and was calculated using the formula.<sup>18</sup>

$$1RM = \text{load} / (1.0278 - 0.0278$$

$$(\text{number of repetition to exhaustion}))$$

## Exercise protocol

All participants participated in the exercise for two consecutive and non-consecutive days from 9 to 11 AM. The total duration of each session lasted about 60–70 min, and there was a one-week rest between the two protocols. After 10 min of special warm up, the participants began to perform circuit exercises. The CRE included bench press, lat pull down, triceps curls, biceps curls, overhead press, vertical row, leg press, leg extension, push up, and sit up with 75% 1RM, 11 repetitions and one-minute recovery between the exercises and 3 min between each round.<sup>7</sup> At the end of each exercise session, subjects were allowed to cool down for 5 min. Blood and salivary samples were taken in the morning of the first exercise session after 12 h of fasting.



**Figure 1** Study design. CRE: Circuit resistance exercise.

Afterwards, samples were taken immediately after exercise at the second exercise session (Fig. 1).

### Biochemical analyses

Blood and saliva samples were taken during baseline and immediately after exercise. Salivary cortisol concentrations were measured in duplicate by using a commercially prepared ELISA kit (Diagnostics Biochem Canada, Inc.) with modified procedures suggested by the manufacturer. A lower limit of detection for saliva cortisol was 1.0 ng/dl. Salivary concentration of IgA was assayed by immunodiffusion method by use of IgA kit (The Binding Site Ltd., Birmingham, UK). The CV for IgA was <3%. Five ml of blood samples of each subject were collected in a 12 h fasting state of the brachial vein in sitting position. CK activity was assayed spectrophotometrically through the use of commercially

available kits (Pars Azmun Co., Tehran, Iran). The CV for CK was <4%.

### Statistical analyses

Descriptive statistics were used to determine the properties of the indicators mean and standard deviation of the participants in terms of age, height, and weight. The SPSS version 20 was used for statistical analysis. After ensuring a normal distribution using the Shapiro–Wilk test, the dependent variables between two groups were compared by paired samples *t*-test. One-way Analysis of Covariance (ANCOVA) was used for group comparison. Effect sizes (ES) were calculated to determine training effects. The magnitude of the ES statistics was considered trivial <0.20; small, 0.20–0.50; medium, 0.5–0.80; large, 0.8–1.30; or very large >1.30.<sup>19</sup> The effect size is reported in conjunction with the 95%

**Table 1** Subject characteristics.

Variables	Mean	Std. deviation
Age (year)	22.25	1.61
Weight (kg)	73.40	3.25
Height (cm)	172.41	5.94
BMI (kg/m <sup>2</sup> )	24.7051	1.44

confidence interval (CI) for all analyzed measures. Significance level was  $P \leq 0.05$  considered.

## Results

The descriptive characteristics of the subjects are reported in Table 1. The results indicate that the levels of cortisol and CK activity increased in two consecutive days of circuit resistance exercise (TCD-CRE) and two non-consecutive days of circuit resistance exercise (TNCD-CRE) groups, and contrary to these, IgA levels decreased in both groups (Fig. 2). Significant changes were observed for concentrations of cortisol, CK and IgA in pre and post-test on TCD-CRE group ( $P < 0.001$ ,  $ES = -0.75$ ,  $95\% CI = -1.45$  to  $-0.19$ ;  $P = 0.001$ ,  $ES = 2.70$ ,  $95\% CI = -0.15$  to  $6.71$ ;  $P = 0.011$ ,  $ES = 0.10$ ,  $95\% CI = -0.09$  to  $0.25$ ; respectively, Fig. 2). Also, a significant change was observed for concentrations of cortisol in pre and post-test on TNCD-CRE group ( $P < 0.001$ ,  $ES = -0.30$ ,  $95\% CI = -0.87$  to  $-0.12$ ). However, there were no significant differences observed in CK and IgA between pre-test and post-tests of the group TNCD-CRE ( $P = 0.24$ ,  $P = 0.11$ ; respectively, Fig. 2).

The result of One-way ANCOVA showed significant differences in CK and cortisol between the TCD-CRE and TNCD-CRE groups ( $P = 0.002$ ,  $P = 0.001$ ; respectively, Table 2).

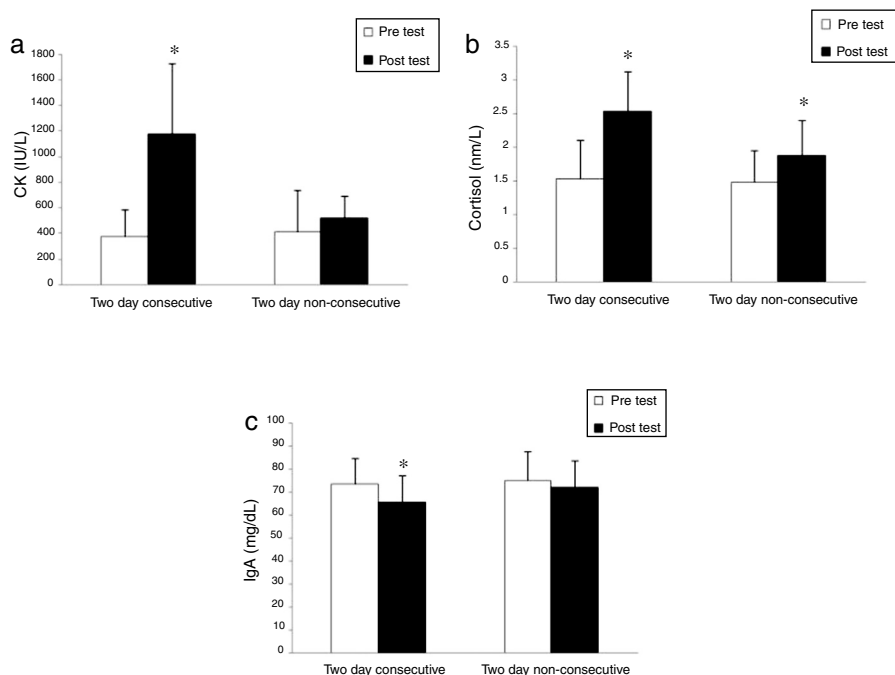
However, no significant difference was found in IgA between the groups ( $P = 0.06$ , Table 2).

## Discussion

The main findings showed that two consecutive sessions of CRE cause significant changes in cortisol, CK and IgA. In addition, the results showed that two non-consecutive sessions of CRE with 48 h of recovery only caused a significant change in cortisol. Moreover, significant changes were observed in cortisol and CK levels between the two groups.

Studies have shown that resistance exercises have the greatest impact on muscle mass and strength, and the greatest effect of these exercises was due to the repetition of the practice with appropriate recovery. Recovery time of 2 or 3 days is the best recovery known to increase the effects of exercises in the similar muscles. Reducing recovery time between sessions leads to accumulation of exercise stress and causes inflammation or catabolic responses.<sup>20,21</sup> However, there is little information available and there is little research on the effects of short-term recovery between sessions on the response of cortisol, CK, and IgA to CRE. Most studies have looked at the rest between the sets on cortisol, CK and IgA.

Participating in competitive encounters causes stress in athletes and may have adverse effects on physiological systems in the body, such as the immune system. The stress caused by competitions by stress hormones reduces a number of defensive factors and increases the risk of infection; ultimately, it reduces the effectiveness and maintenance of athletic performance and increases the risk of injury. Researches have shown that cortisol levels increase after resistance training, especially by using multiple sets with



**Figure 2** Changes in CK (a), Cortisol (b) and IgA (c) following two day consecutive and non-consecutive CRE (mean + SD). CK: creatine kinase; IgA: immunoglobulin A; CRE: circuit resistance exercise. \*Denotes significant differences between pre and post test values ( $P \leq 0.05$ ).

**Table 2** Results of ANCOVA.

P	F	Mean diff	Std. error	Variable
0.002*	13.29	666.77	182.83	CK (IU/L)
0.001*	17.63	0.618	0.147	Cortisol (nm/L)
0.06	3.83	5.58	2.85	IgA (mg/dl)

CK: creatine kinase; IgA: immunoglobulin A; ANCOVA: analysis of covariance.

\*  $P \leq 0.05$ .

short periods of 60 to 120s of rest.<sup>22,23</sup> Peak plasma cortisol occurs immediately after physical activity, and remains above the baseline 30 min after physical activity, begins to decrease to the baseline level after 30–60 min of physical activity.<sup>22,24</sup> 90–120 min after physical activity, cortisol returns to baseline levels or lower.<sup>7</sup> This is an important time as neutrophils and monocytes are similarly increased during this interval, and several studies have shown a correlation between cortisol changes and the subset of leukocytes after resistance training. Moreover, this is important for clarifying the effect of cortisol on redistribution of leukocytes.<sup>7</sup>

The findings of the present study indicated a significant increase in cortisol levels in both groups. Furthermore, the observed changes between the two groups were significant. These changes were meaningful and greater for the TCD-CRE groups. It has been suggested that cortisol is responsible for reducing lymphocytes during recovery.<sup>4</sup> Ramel et al.<sup>7</sup> argued that cortisol concentrations had a negative correlation with all lymphocyte subunits (i.e. T-helper, T-suppressor and NK cells) during recovery. Reduction of CD4/CD8 after exercise is associated with increased cortisol concentrations. In this regard, Leite et al.<sup>25</sup> investigated the effect of two separate sessions of resistance training with different exercise volumes (three set of 6 and 12 repetitions, with 80% 1RM, with two minutes of rest and a one-week interval) on the peripheral blood cortisol of men. Their results indicated a significant increase in cortisol levels following exercise with 12 and 6 RM. Moreover, Simao et al.<sup>26</sup> reviewed the effect of resistance training (three times with 70% 1RM and two minutes of inactive recovery) with two types of involved muscle sequences on hormonal responses. Their results showed no difference in the response of cortisol following two different muscle sequences. In addition, Uchida et al.<sup>27</sup> have studied the effect of different resistance training programs on cortisol responses. Their results indicated that cortisol had little variation due to resistance training. Moreover, they argued that the training program with 75% 1RM stimulated cortisol. Crewther et al.<sup>28</sup> concluded that increased cortisol concentrations were related to the total volume of resistance training (number of sets  $\times$  volume). Moreover, Mulligan et al.<sup>29</sup> indicated a greater increase in cortisol after multiple sets versus single-set resistance training. In general, acute cortisol elevation has been observed after resistance training involving multiple exercises or a large muscle mass.<sup>27–29</sup>

Contrary to these results, Mohebbi et al.<sup>30</sup> observed a significant reduction in serum cortisol levels after exercise, 3h after 4 consecutive days of training sessions, as well as 3h after 4 non-consecutive days of CRE. Smilios et al.<sup>31</sup> and Hough et al.<sup>32</sup> showed no significant increase in cortisol concentrations of their subjects after increasing the

number of sets from 4 to 6 and after 8 sets with 10 RM of squat exercises. They argued that resistance exercises with a rest period of 2 to 3 min or more between sets, but generally did not cause a significant increase in cortisol. Heavy resistance exercise strongly activates the sympathetic nervous system. The sympathetic nervous system is heavily linked to the function of the immune system, with innate immune cells expressing both  $\alpha$ - and  $\beta$ -adrenergic receptors and T-cytotoxic and B-lymphocytes expressing  $\beta_2$ -adrenoreceptors. Considering lack of findings on the effect of cortisol, the hormonal environment seems to favor activation of the sympathetic nerve relative to the effects of cortisol mediation after resistance training.<sup>33</sup>

The results of this study indicated that CK activity increased in both groups, but only changes in the exercise group for two consecutive days were significant. Moreover, changes in CK were significant between the two groups. In this regard, Heavens et al.<sup>34</sup> studies showed a significant increase in CK through resistance exercise. Ribeiro et al.<sup>35</sup> and Mayhew et al.<sup>36</sup> showed that serum CK levels increased significantly in 24 to 48h after exercise with a 1–3 min rest interval between sets. Furthermore, Callegari et al.<sup>37</sup> showed that bi-set sessions and multiple sets of resistance exercise increase the levels of CK, but the levels of CK are lower compared to the aerobic exercise. Moreover, Machado et al.<sup>11</sup> concluded that serum CK levels increased 24–72h after each resistance exercise session. Mechanical stresses applied by 4 sessions of resistance exercise create similar muscle fibers injuries that are independent of rest periods (60, 90, 120 and 180s) between sets. They argued that the overall amount of exercise was the main determinant of muscle damage in trainees who were accustomed to short-term resistance exercises.

As stated above, CK is a free intramuscular protein in the circulation during muscular damage and is cleared from the blood by the reticuloendothelial system.<sup>38</sup> CK is a known marker, but its quality response is significantly different.<sup>38,39</sup> Additionally, CK is associated with damage caused by mechanical stimuli and affects many blood concentration factors. In males and females, peak concentrations are 24h which is consistent with other studies.<sup>11,36</sup> Women have always shown lower levels of CK compared to men. This may be related to the higher muscle mass in men, so, most muscle fibers may be mechanically damaged and release such proteins.<sup>39</sup> The true responses of CK to exercise are related to factors such as age, type of muscle fiber, muscle group, and movement velocity<sup>11</sup>; all these can help explain the different results of published studies. Regarding the above mentioned factors, it seems that CRE leads to a greater increase in CK due to the use of the main muscle groups and the high training volume and, if there is no proper

recovery during the 24 h after the exercise, it increases more than the recovery exercise sessions with 48 h.

Our results showed a decrease in IgA levels in both groups, but only changes in two consecutive days of exercise group were significant. Also, no significant changes were observed between the two groups. Several studies have shown that heavy exercises can reduce salivary IgA (s-IgA) levels after exercise.<sup>40,41</sup> The proposed mechanism for reducing s-IgA involves a change in IgA transport across the mucosal epithelium, or the sympathetic mediation of vasoconstriction in the oral submucosa, thereby reducing the migration of cellular synthesis and IgA secretion.<sup>42</sup> Bay et al.<sup>17</sup> investigated acute and chronic immune responses to resistance exercises in consecutive and non-consecutive days and showed that there was no significant difference in immune parameters between the two exercise groups and both had similar responses. However, high-intensity exercises cause a significant reduction in muscle function and recovery. This may be due to the use of more type II muscle fibers in high-intensity eccentric exercise, which is known to be more sensitive to disorders than type I muscle fibers.<sup>43,44</sup>

In contrast, Potteiger et al.<sup>45</sup> and Nunes et al.<sup>26</sup> showed that there was no significant change in IgA concentration after resistance exercise in untrained and untrained women. It has been suggested that trained women need greater stimulus for the response and secretion of these immune parameters compared to older untrained women. Moreover, some studies showed that physical activity does not alter IgA, or increases IgA after activity.<sup>46</sup> The amount of IgA flow and its secretion per day varies, and current immune levels may be contributing to exercise-induced IgA responses.<sup>42,46</sup> It has been shown that resistance exercises strongly modulate other immune system performance markers (e.g., white blood cells, T-cells, leukocytes, lymphocytes), some of which occur in the absence of any change in IgA.<sup>45</sup>

## Conclusions

In general, the results of this study showed significant changes in levels of cortisol, CK and IgA in the exercise group for two consecutive days. It seems that in CRE, it is necessary to have a minimum 48-h recovery between the sessions due to the activity of the main muscle groups and the high volume of exercise, so these exercises will not disrupt the immune system.

## Ethical approval

“All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

## Conflicts of interest

The authors declare no conflict of interest.

## Acknowledgments

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