UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

Analysis of DOMS indicators after performing a sub-maximal repetition strength training protocol exercise: Influence of exercise order

Análise de indicadores de dor muscular tardia após a realização de um protocolo de exercícios de força com repetições submáximas: Influência da ordem dos exercícios

DOUTORAMENTO EM CIÊNCIAS DO DESPORTO

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Abstract

Strength Training (ST) has been recommended as part of several physical training programs aimed to improve health and quality of life. The exercises' order (EO) seems to have a great importance in the development of a ST program. ST and eccentric movements tend to produce more damage related to structural changes in skeletal muscle fibers especially due to the mechanical and metabolic stimulation. These damages can increase the metabolism associated with increased oxidative stress and/or damage to the vascular epithelium, inducing an increase of xanthine oxidase activity (XO). The sarcolemma damage may also increase the delivery of cytokine into blood stream that would augment inflammatory response, and consequently oxidative stress damage. Altogether these changes commonly induce delayed onset of muscle soreness (DOMS). The exercises execution order is a variable that has been addressed in the literature, but up to now, there are no studies relating muscle damage, oxidative stress and ST program with distinct orders. The objectives of this study were: (i) to investigate the role of exercise order on total number of repetitions and to evaluate the possible importance on muscle damage on the rating of perceived exertion; (ii) to investigate if one regular high intensity ST session performed to voluntary fatigue could cause muscle damage and similar DOMS responses at multiple time points and; (iii) to check if a session of ST with sub-maximal repetitions induces systemic oxidative stress, measured by lymphocytes DNA damage. Ten healthy trained men completed two sequences: sequence A (SEQA) - leg press (LP), leg extension (LE), leg curl (LC), bench press (BP), shoulder press (SP) and triceps extension (TE); and sequence B (SEQB) - was executed in reverse order. The strength training session consisted of 20 repetitions of the first exercise at 40% of 1RM to warm-up, followed by sequence training. All exercises were performed in three sets to volitional fatigue at 80% of 1RM with two minutes rest intervals of passive recovery between sets and exercises. All of the subjects had their capillary blood samples collected for the creatine kinase (CK) and lymphocytes DNA damage measure, and DOMS measured before, immediately after and 24, 48 and 72 hours after the exercise session. The subjective effort perception was measured by OMNI-RES scale before exercise and at the end of each exercise.

There were significant ($p<0.05$) differences between SEQA and SEQB in the total number of repetitions for TE, LE and LC. The highest CK concentrations were observed 24h after both sequences, but no differences were found at any time between them, revealing that muscle damage occurred independent of the exercise order. The CK concentration was highest at post and 24h after ($p<0.05$), when compared to pre exercise condition and decreased at 48h ($p<0.05$) and at 72h ($p<0.05$) post exercise compared to 24h. The RPE was higher in TE in SEQA, ($p<0.05$) and LP in SEQB ($p<0.05$). The DOMS values were higher 24h post exercise ($p<0.05$) in relation to pre exercise and decreased after 72h. Significant differences in DOMS were also found between 24h and 72h ($p<0.05$) and between 48h and 72h ($p<0.05$) after the exercise. DNA results revealed significant differences in the damage of DNA strand breaks ($p<0.05$) between 24h and 48h after the ST session, and also revealed significant differences in DNA FPG sites between 24h and 48h ($p<0.05$) and between 24h and 72h after exercise ($p<0.05$). We concluded that EO affects the number of repetitions and perceived exertion. These study results also indicate that whenever one exercise is the last of a sequence performed in a training session, its number of repetition could be negatively affected. This was confirmed whether it was a large or small-muscle group exercise and could not be explained by differences in muscle damage. Moderate intensity ST session performed to voluntary fatigue induced an increase in CK concentrations and it was not related to EO. DOMS scale could be used in training routines to appreciate skeletal muscle damage and the whole body response to ST at 24h after. There was a trend of increasing both CK and DNA at 24h after the ST session, revealing the existence of muscle damage as well as lymphocytes DNA damage possibly caused by oxidative stress.

KEYWORDS: strength training, exercise order, muscle damage, creatine kinase, OMNI-RES, DOMS scale, comet assay, oxidative stress.

Resumo

O treino de força tem sido recomendado como parte de um programa de treino com o objetivo de melhorar a saúde e a qualidade de vida. A ordem dos exercícios parece ter uma grande importância no desenvolvimento de um programa de treino de força. O treino de força e os movimentos excêntricos tendem a produzir mais danos relacionados às mudanças estruturais nas fibras musculares esqueléticas especialmente devido ao estimulo mecânico e metabólico. Esses danos podem elevar o metabolismo associado ao aumento de estresse oxidativo e/ou dano no epitélio vascular, incluindo um aumento da atividade da xantina oxidase. O dano no sarcolema também pode gerar um aumento das citocinas na corrente sanguínea o que pode acrescentar uma resposta inflamatória e consequentemente o dano por estresse oxidativo. De um modo, geral essas modificações frequentemente induzem a dor muscular tardia (DOMS). A ordem de execução dos exercícios é uma variável que tem sido estudada, mas até então não há estudos relacionando dano muscular, estresse oxidativo e treino de força com ordens distintas. Os objetivos deste estudo foram: (i) investigar o papel da ordem dos exercícios no número total de repetições e a possível importância no dano muscular e na percepção de esforço; (ii) investigar se uma sessão de treino de força de alta intensidade realizada até a fadiga voluntária poderia causar respostas similares nos danos musculares e DOMS e (iii) verificar se uma sessão de treino de força com repetições submáximas induz a um estresse oxidativo sistêmico, medido pelos danos de DNA de linfócitos. Dez homens treinados realizaram duas sequencias: sequencia A (SEQA) – pressão de pernas (LP), extensão de pernas (LE), flexão de pernas (LC), supino (BP), pressão de ombros (SP) e tríceps de pé (TE); e a sequencia B (SEQB) que foi realizada na ordem inversa, tendo inicio com os membro inferiores. A sessão de treino de força consistiu de 20 repetições do primeiro exercício com carga de 40% de 1RM para aquecimento, seguida da realização da sequencia dos exercícios. Todos os exercícios foram realizados em três séries com carga de 80% de 1RM até a fadiga voluntária, dois minutos de intervalo de recuperação passiva foi dado entre as séries e os exercícios. Foi feita a coleta de sangue capilar para todos os sujeitos para a análise da CK e dos danos de DNA de linfócitos, a DOMS foi

medida antes, imediatamente após e 24, 48 e 72 horas após a sessão de treino de força. A percepção de esforço foi avaliada pela escala de OMNI-RES antes do exercício e no final de cada exercício. Houve diferença significativa ($p<0.05$) entre a SEQA e a SEQB no número de repetições para TE, LE e LC. A maior concentração de CK foi observada às 24h após o exercício nas duas sequencias, contudo nenhuma diferença foi encontrada em qualquer um dos momentos, revelando que o dano muscular ocorreu independente da ordem dos exercícios. A concentração de CK foi maior imediatamente após e 24h após ($p<0.05$) a realização do protocolo, quando comparada com as condições pré-exercício e diminuiu nas 48h e 72h ($p<0.05$), após o protocolo quando comparada com 24h. A PSE foi maior para o exercicio de TE na SEQA, ($p<0.05$) e para o LP na SEQB ($p<0.05$). Os valores da DOMS foram maiores nas 24h após o exercício ($p<0.05$) em relação aos valores pré-exercício e diminuiram após 72h. Diferenças significativas na DOMS também foram encontradas entre 24h e 72h ($p<0.05$) e entre 48h e 72h ($p<0.05$) após o exercício. Os resultados de DNA revelaram diferenças significativas nos danos de DNA basais ($p<0.05$) entre 24h e 48h após o treino de força e também se constatou diferenças significativas nos danos de DNA FPG sites (por estresse oxidativo) entre 24h e 48h ($p<0.05$) e entre 24 e 72h após o exercício ($p<0.05$). Foi concluído que a ordem dos exercícios afeta o número de repetições e a percepção do esforço. Os resultados deste estudo sugerem que sempre que um exercício é o último a ser realizado em uma sessão de treino de força, o seu numero de repetições será negativamente afetado. Uma sessão de treino de força de intensidade moderada realizada até a fadiga voluntária induz a um aumento da CK independente da ordem dos exercícios. A escala de DOMS pode ser utilizada nas rotinas de treino para avaliar o dano muscular esquelético e a resposta geral ao treino de força nas 24h após a sessão. O aumento da CK e dos danos de DNA de linfócitos expressam a mesma tendencia de variação. Uma sessão de treino de força pode gerar danos no músculo esquelético e sistémicos causados pelo estresse oxidativo.

Palavras chave: treino de força, ordem dos exercícios, danos musculares, creatina quinase, OMNI-RES, escala de DOMS, ensaio do cometa, estresse oxidativo.

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List of Abbreviations

1RM test - one repetition maximum test

BP - bench press

CK- creatine kinase

DNA FPG Sites - DNA oxidative stress damage

DNA Strand Breaks - DNA basal damage

DOMS – delayed onset muscle soreness

LC - leg curl

LE -leg extension

LP - leg press

OS- oxidative stress

PSE – perceived exertion

RPE - rating perceived exertion

SEQA - sequence A

SEQB - sequence B

SP- shoulder press

ST – strength training

TE- triceps extension

1. GENERAL INTRODUCTION

1. General Introduction

Through different forms of activity, the American College of Sports Medicine (ACSM) recommends exercises guidelines for different types of population (ACSM, 1998, 2001, 2002, 2009). Among these activities is strength training (ST) whose first revision, addressing their methodological variables, was published in 2002 (ACSM, 2002). ST has been recommended as part of several physical training programs and has been considered in conditioning programs aimed to improve health and quality of life (ACSM, 2009). ST promotes numerous health benefits, improving capacities related with motor performance, such as speed, agility, balance, coordination and flexibility, favoring various sports (ACSM, 2002, Williams et al., 2007; ACSM, 2009). ST results in countless adaptations such as muscle hypertrophy, improvement in neural response, increases recruitment of motor units, increases and improves muscle power, gain lean mass, increases muscle strength, improves muscular endurance, decreases body fat and improves sports performance (ACSM, 2002, 2009; Carroll et al., 2001; Komi, 2003).

In 2009, the ACSM published its second review considering the variables that must be considered in a strength training program. These are: number of sets, number of repetitions, load intensity, rest period between sets and between training sessions, choice of exercises, muscle action, order of exercises, running speed, weekly training and recovery time (ACSM, 2009).

It is important to notice that the benefits of ST can only be attained if the manipulation of these variables occurs in an appropriate manner. The intensity and volume' training should be equalized to provide stimuli according to the desired objective and to induce neuromuscular and morphology changes. The exercises' order seems to have a great importance in the development of a ST program. The exercises sequence can significantly affect the total number of repetitions in a training session and consequently the strength and muscle hypertrophy (Sforzo y Touney, 1996, Simão et al., 2002, Simão et al., 2005, Spreuwenberg et al. 2006, Simão et al., 2007; Spineti, et al. 2010). There are evidences that when the exercises for larger muscle groups are ordered at the beginning of the session, a greater volume of training is produced (Sforzo y

Touney, 1996, Simão et al., 2005, Simão et al., 2007). According to the ACSM, in the positioning for ST (ACSM, 2002, 2009), the recommendation is that the ST program should start with exercises for the major muscle groups and continue within the same session with exercises for smaller groups. However, in the past 10 years there is no consensus about it.

In 1996, Sforzo and Touey showed that the exercises order generated a different response to ST. These authors compared two sessions of ST with different exercises orders. The ordering of the chosen exercises consisted of upper and lower limbs muscle groups involving uni and bi-articular exercises. The first session began with the larger muscle groups and give continuity to smaller groups while the second session was held in reverse order. This study showed that while exercising the smaller muscle groups before large, the total output power of the large ones was smaller. In relation to higher total output power, this would occur only in some exercises, when performed early in the ST session. This study was the first to demonstrate that in ST the order in which the exercises are performed showed different responses, and may have an influence on performance throughout the ST session. Since 2005, several studies (Simão et al., 2005; Spreuwenberg et al., 2006; Gentil et al., 2007, Simão et al. 2007; Farinatti et al., 2009, Rodrigues et al., 2010) on the order of the exercises were published showing the importance of exercise order in a training session, opening up as a result a new line of research, without scientific support, so far. The order of exercise began to be investigated in several ways, for example, order and perceived exertion (Simão et al., 2005; Spreuwenberg et al., 2006; Simão et al., 2007), order of exercises in women, (Simão et al., 2007), order and number of repetitions (Simão et al., 2007), order of exercises in upper limb exercises (Simão et al., 2005, Gentil et al., 2007), order of exercises and oxygen consumption (Farinatti et al., 2009), order of exercises and neuromuscular activity (Brennecke et al., 2009), order of exercises and blood lactate, perceived exertion and affective response to training (Bellezza et al., 2009), order of exercises and maximum force (Dias et al., 2010), order of exercises, maximum strength and muscle volume (Simão et al., 2010; Spineti et al., 2010) and exercise order and interval between sets (Miranda et al., 2010).

Simão et al. (2005) have verified the influence of the order of upper limb exercises and perceived exertion (PSE) in 14 men and four women trained in ST in two identical and reverse sequences with loads for 10RM in five exercises. Researchers have observed a decrease in the number of repetitions in the exercises that were conducted at the end of the program, showing that regardless of the size of the muscle group, the groups that are at the end are negatively affected in its total number of repetitions. The PSE between the sequences showed no significant difference, however, showed a tendency to be higher when the series was started by large muscle groups. In another study by Simão et al., (2007), with only trained females, using the PSE and an exercise protocol with loads for 80% 1RM for upper and lower limbs, it was observed that the performance of the exercises performed at the end of the series was also lower compared to the same exercise performed at the beginning of the program. However, the PSE showed no significant changes between the sequences. Studies of Sforzo and Touney (1996), Simão et al. (2005) and Simão et al. (2007), corroborate this conclusion showing results that exercises performed at the end of the program are more impaired in their performance, regardless of the size of the muscle group. They also provide evidence that a training session should be started with the exercises of the highest priority to the individual, regardless of the size of the muscle group. According to these authors, it appears that the performance decrease of exercise executed at the end of the session is due, in part, to muscle fatigue that accumulates along the session and results in lower performance. Despite the PSE did not differ significantly in the aforementioned studies, there is a tendency to be greater in exercise protocols that start with large muscle groups (Simão, et al., 2002).

As already seen, the execution order of the exercises is a variable that has been addressed in the literature, but until now no study to our knowledge related muscle damage and a ST program with distinct orders. This is an important aspect that must be considered in practice and that may contribute to the development of more efficient and safe strength training programs.

Markers of muscle damage can be managed through the high activity of creatine kinase (CK) (Mc Hugh, et.al., 1999), increased myoglobin, increased hydroxyproline and creatinine elevation (Armstrong, 1984). These may also

result from metabolic overload, such as the formation of reactive oxygen species (ROS) (Bloomer et al. 2005), imbalance in the calcium release (Armstrong, 1984; Mc Hugh, et.al., 1999), hyperkalemia (Armstrong, 1984) as well as mechanical causes such as damage to the line Z with resulting rupture or damage the sarcolemma (Mc Hugh, et.al., 1999) and connective tissue injury (Armstrong, 1984). The products of oxidative damage tend to arise more frequently after prolonged exercise than after ST. The ST and eccentric movements tend to produce more damage related to structural changes in muscle fibers due to the mechanical traction (Mc Hugh, 1999). Besides the mechanical traction, some damage may also occur resulting from an increased metabolism associated with increased oxidative stress and / or damage to the vascular epithelium similar to those disclosed in situations of ischemia and reperfusion, inducing an increase of xanthine oxidase (XO) (Hellsen et al. 1997; Fisher-Wellman and Bloomer, 2009). This XO increase is associated with the inflammatory process, and can be induced by ischemia that occurs in these types of exercises and by leukocytes infiltration (Hellsen et al. 1997).

The increase of CK, lactate dehydrogenase (LDH), troponin can also be indicators of muscle damage caused by mechanical traction, more common in strength training, and may also arise in the prolonged and intense exercise (Fisher-Wellman and Bloomer, 2009).

Creatine Kinase is present in various tissues such as brain, cardiac muscle, skeletal muscle, prostate, intestine, uterus, placenta, thyroid, lung and bladder. In skeletal muscle and heart reveals an increase in membrane permeability and damage of these tissues (Nosaka & Clarkson, 1995; Lee, et al. 2002; Cheung et al. 2003). Some authors have used CK to quantify muscle damage after ST exercise protocols (Kleiner et al., 1996; Jamurtas et al., 2005; Paschalis, et al., 2005). Such protocols would generate a line Z rupture and a sarcolemma lesion allowing the diffusion of enzymes such as the CK into the interstitium (Cheung et al. 2003). The migration of CK from the cell can produce an increase in CK from 100 to 40,000 U/L characterizing muscle injury (Cheung et al. 2003). Whereas there is a tendency (Sforzo y Touney, 1996, Simão, 2005, Simão, 2007) to perform a higher strength volume when the major muscle groups are exercised at the beginning of the session, it is possible that the order of

exercises influence muscle damage associated with exercise. Being muscle damage an important part that occurs in training adaptation, exercise order may have, in fact, a decisive role in shaping the sessions of ST. Thus, we intend to study if the exercises order execution in a strength protocol with two different sequences alters the concentrations of blood CK and perceived exertion after different times of the exercises completion.

Whereas assessment of muscle damage using biochemical indicators is not accessible to most coaches, it becomes important to find other alternative methodologies for the purpose. Another way to assess muscle damage associated with ST is through the scale of delayed onset muscle soreness (DOMS), a visual analogue scale (VAS) of pain perception (Price et al., 1983; Clarkson et al., 1992; Cleather et al., 2007), from zero to 100, where zero indicates no pain and 100 the worst possible pain. DOMS is a type I muscle injury characterized by muscle pain and other symptoms that appears approximately 12 to 72 hours after workout or intense or unusual exercise and can still occur due to the mechanical stress that occurs in training of high magnitude (Armstrong, 1984; Szymanski, 2001; Cheung et al. 2003; Zainuddin et al. 2005; Cleary, et al. 2006; Nosaka et al., 2007) and may remain five to seven days (Szymanski, 2001). The signs and symptoms presented in DOMS are: diffuse pain, discomfort to the touch, stiffness, discomfort, reduced muscle power and peak torque, decrease or loss of muscle strength on muscle that was exercised, decreased range of motion, change on motor units recruitment pattern and decreased neuromuscular function (Proske & Morgan, 2001; Szymanski, 2001; Cheung et al., 2003; Zainuddin et al., 2005, Cleary et al., 2006; Nosaka et al., 2007).

Several theories have been proposed to explain the pain associated with DOMS, some of them are damage to muscle structure (Friden & Lieber, 1992), muscle spasm, connective tissue damage, muscle damage, inflammation, enzyme efflux, altered blood flow, infiltration of inflammatory factors such as neutrophils and monocytes (Armstrong, 1984; Friden & Lieber, 1992; Smith, 1991; Cheung et al. 2003), as well as the production of ROS, resulting in increased oxidative stress that can also produce muscle damage (Proske & Morgan, 2001). It is known that during the ST various aggressions muscle can

occur due to the mechanical stimulation. These attacks often become necessary to promote physiological responses expected from a ST program. Such damage can last for several hours and even days after the effort. However, in gymnasiums it becomes impracticable to quantify the damage through biochemical markers. Regarding subjective damage indicators, it becomes easier to use, since they have lower cost, are easy to use and don't use invasive techniques. In this sense, there has been an attempt to associate DOMS to the modification of some blood markers such as CK (Brown et al. 1997, Dolezal et al. 2000, Lee et al. 2002; Cheug et al. 2003, Zainudin et al. 2005). However, the literature is poor showing studies that used subjective and biochemical markers of muscle damage after a ST protocol, as used in the daily life of gymnasiums. Another aspect that may also affect DOMS is the order of the exercises. As previously mentioned, the order of exercises has been studied in relation to several variables, but no study has addressed the order of exercises and subjective and biochemical damage markers.

Together, muscle and vascular damage induce an increased immune response that will aggravate muscle damage hours (days) after the workout. These immunological changes are accompanied by an increase in oxidative damage in various cells, in particular increased DNA damage. However, with regard to the ST, there are few studies of this nature. Thus, it seems appropriate to consider how far a session of strength training induces DNA damage in lymphocytes and how these injuries are related to muscle damage.

Thus, the objectives of this study are to investigate the influence of a session of strength training with sub-maximal repetitions in blood and subjective markers of muscle aggression, and verify the influence of muscle damage associated with ST in markers of oxidative DNA damage in lymphocytes. This study will be subdivided in the following three studies:

Influence of Exercise Order on Muscle Damage - The first study verifies if the execution order of the exercises in a strength protocol with two different sequences alter the concentrations of CK, perceived exertion and presence of DOMS after different times of the exercises.

Is there a relation between muscle damage and DOMS after a strength training session? - The second study sets out to investigate the relationship

between subjective indicators of DOMS and the presence of muscle damage after several moments of realization of a sub-maximal strength training protocol.

Modification of lymphocyte DNA damage after a session of strength training with sub-maximal repetitions - The third study checks if a session of strength training with sub-maximal repetitions induces lymphocytes DNA damage and how these injuries are related with muscle damage.

Finally, general conclusions of the thesis and some implications for the strength training at gyms will be presented. There will also be some suggestions for future studies.

2. INFLUENCE OF EXERCISE ORDER ON MUSCLE DAMAGE

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Influence of Exercise Order on Muscle Damage During Moderate-Intensity Resistance Exercise and Recovery

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ABSTRACT

This study aimed to investigate the role of exercise order on total number of repetitions and to evaluate the possible importance on muscle damage on the rating of perceived exertion (RPE). Ten trained participants completed two sequences: sequence A (SEQA) was leg press (LP), leg extension (LE), leg curl (LC), bench press (BP), shoulder press (SP) and triceps extension (TE) and in sequence B (SEQB) the order of execution of the exercises was reversed. Highest creatine kinase (CK) concentrations were observed 24 hours following both sessions, but no differences were found at any time between them, revealing that muscle damage has occurred. There were significant differences between SEQA and SEQB in the total number of repetitions for TE, LE and LC. Our results suggest that differences in total strength production when exercise order is changed must be explained by some other mechanisms besides muscle damage and RPE.

KEYWORDS: strength training, physical activity, blood test, ratings of perceived exertion.

INTRODUCTION

Strength training can increase muscle size, increase strength and improving the functional performance (Fröhlich et al 2010, Marks 1996). One of the key variables in strength exercise prescription involves the order in which exercises are programmed during a workout. This can determine the effectiveness of a workout in accomplishing different training goals (Miranda et al 2010). The American College of Sports Medicine (ACSM) position stand on progression models in resistance training for healthy adults (ACSM 2009) recommends that large-muscle group exercises should be performed earlier in a training session. Sforzo and Touey (1996) reported greater total force production in some, but not all, single joint exercises when they were performed early in a training session composed of both upper and lower body large-muscle (multi-joint) and small-muscle (single-joint) group exercises. Therefore, the traditional recommendation regarding exercise order has been to perform multi-joint exercises prior to single joint exercises allowing a greater volume of work (load x repetitions) compared to when single-joint exercises are performed first (ACSM 2009, Sforzo and Touey 1996).

Recently, several studies (Bellezza et al 2009, Brennecke et al 2009, Dias et al 2010, Farinatti et al 2009, Gentil et al 2007, Simão et al 2005, Simão et al 2007, Simão et al 2010, Spineti et al 2010, Spreuwenberg et al 2006) observed that exercise order influences the number of repetitions independently of the muscle group size. In fact, when the exercise was performed at the beginning of the training session, additional repetitions were performed. Considering these results, several studies (Dias et al 2010, Gentil et al 2007, Simão et al 2005, Simão et al 2007, Simão et al 2010, Spineti et al 2010) recommended that exercises that demand maximal adaptation should be placed at the beginning of an exercise session despite the size of the muscle groups involved.

Although differences exist between the total numbers of repetitions when strength training exercise order is changed, to our knowledge, no plausible explanation has been pointed out. Considering that highly fatiguing strength training protocols, such as those that involve moderate intensity sets performed to voluntary exhaustion, may induce significant damage to muscle fibers (Clarkson et al 2006), we speculate that difference in total number of repetitions

could be explained by differences in muscle damage. The level of rating perceived exertion (RPE) at the end of a session could also explain this fact as in this moment any effort could be over assessed.

Serum Creatine Kinase (CK) concentrations have been used as a marker of muscle damage following strength training and may indicate the status of the muscle cell membranes (Clarkson et al 2006, Ferri et al 2006, Jamurtas et al 2006, Paschalis et al 2005, Rodrigues et al 2010). Therefore the purpose of the current study was to compare CK concentrations and RPE at multiple time points after strength exercises that incorporated different orders exercise. We have hypothesized that different exercise orders would evoke divergent CK and RPE responses.

METHODOLOGY

Experimental Approach to the Problem

To investigate the effect of two different exercise orders on CK and RPE, the one repetition maximum test (1RM test) had to be assessed in order to determine 80% of 1RM load per subject and for each exercise. The figure 1.1 depicts experimental protocol design.

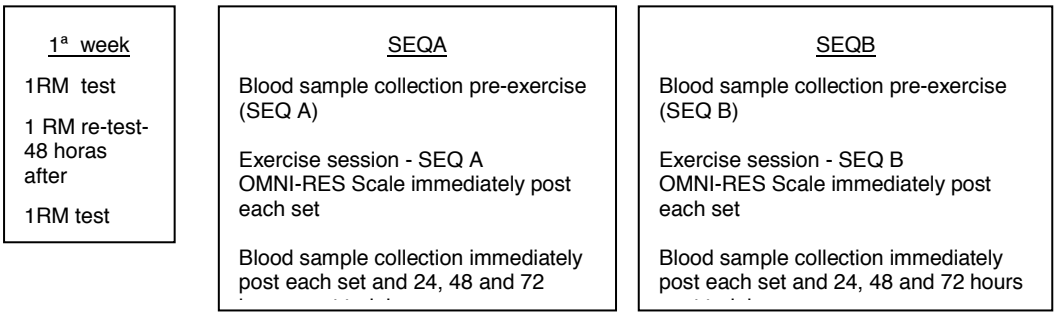

FIGURE 1.1 The experimental protocol design in a counterbalanced crossover design

The two sessions were composed by the same exercises performed in different orders. Sequence A (SEQA) began with large-muscle group exercises and progressed toward small-muscle group exercises. The exercise order for SEQA was leg press (LP), leg extension (LE), leg curl (LC), bench press (BP), seated machine shoulder press (SP), and triceps extension (TE). Sequence B (SEQB) began with small-muscle group exercises and progressed toward large-muscle group exercises. The exercise order for SEQ B was LC, LE, LP, TE, SP and

BP. All exercises in both sequences were performed in three sets to volitional fatigue with 80% of 1RM load per subject and for each exercise. Sets and exercises in both sequences were separated by two minutes rest intervals of passive recovery. The total number of repetitions performed was determined for each set of each exercise for both sequences.

Subjects

Ten trained men (24.50 ± 4.95 yrs; 74.20 ± 7.78 kg; 1.77± 0.09 cm; 23.75 ± 0.2 $kg.m^2$, Mean± SD) participated in the study. Inclusion criteria consisted of the following: a) all subjects must have had at least two years of recreational strength training experience performing all selected exercises; b) did not have any medical conditions that might be aggravated by participation in the current study; and c) did not use any nutritional supplements or ergogenic aids. All subjects read and signed an informed consent document and were asked to avoid any kind of any strength training a week before and during the current study. Experimental procedures were in accordance to the Declaration of Helsinki and the study protocol was approved by the Research Ethics Committee of the Institution.

One Repetition Maximum Testing (1RM test)

The 1RM tests were performed on two nonconsecutive days (separated by 48 hours) from which test-retest reliability was calculated (Simão et al 2005). The 1RM was determined in fewer than five attempts with a rest interval of five minutes between attempts and 10 minutes between assessments for different exercises. The 1RM tests were performed in a counterbalanced order. The heaviest load achieved on either of the test days was considered the 1RM. To minimize possible errors in the 1RM tests, the following strategies were adopted (Simão et al 2005): a) all subjects received standard instructions on the general routine of data assessment and proper technique for each exercise before testing; b) the exercise technique of subjects during all testing sessions was monitored and corrected as needed; c) all subjects received verbal encouragement during testing. The mass of all weight plates and bars used for measuring 1RM was determined with a precision scale and all machine

exercises were performed on an Image Sport Machine® (Spain). The same range of motion was used to define a successful repetition for each exercise.

Training Sessions

One week after the 1RM test had been determined the subjects performed one of the two exercise sequences (SEQA or SEQB) in a counterbalanced crossover design. In the following week subjects performed the other exercise sequence. Warm-up session before each exercise sequence consisted of 20 repetitions in the first exercise of the session (LP for SEQA and LC for SEQB) at 40% of the 1RM load. A two minutes rest interval was allowed after the warm-up set before subjects performed the assigned sequence. Both exercise sequences consisted of three sets of each exercise to failure with 80% of 1RM load with two minutes rest intervals between sets and exercises.

During exercise sessions, subjects were verbally encouraged to perform all sets to concentric failure. No pause between the eccentric and concentric phases of each repetition was allowed. The total number of repetitions for every set of every exercise was recorded. Strength training sessions of individual subjects were performed in the morning and approximately at the same time of the day.

Creatine Kinase

To analyze blood CK activity (U/L) capillary blood samples were collected into 80 µL heparinized capillary tubes (Cat nº 955053202 Reflotron®). After the harvest, the blood samples were identified for subsequent use. Serum CK concentrations were determined with 32 µL of blood sample on the Reflotron® Plus – Roche, in which specific reagents (Cat nº 1126695 Reflotron®) were used to analyze the CK concentrations. All blood samples were collected at multiple time points that included: before the bout, immediately after the bout and at the 24^{th}, 48^{th} and 72^{nd} hours after training.

Rating of Perceived Exertion

The RPE was determined using the OMNI-RES scale, which was design specifically for strength training (Lagally and Robertson 2006). All subjects performed a familiarization with the OMNI-RES Scale, two weeks before 1RM

tests sessions. Prior to the warm-up in the experimental sessions, the scaling instructions for the OMNI-RES Scale were reminded to each subject in order to assess perceived exertion. The OMNI-RES was showed to the subjects immediately after each set of each exercise.

Statistical Analyses

Intra-class correlation coefficients (ICC) were used to determine 1RM test-retest reliability. Two-way ANOVAs with repeated measures were used to compare differences in CK concentrations between SEQA and SEQB at multiple time points and the total number of repetitions completed for each exercise and each set individually between SEQA and SEQB. The Wilcoxon test was used to compare the RPE for each exercise between SEQA and SEQB. An alpha level of 0.05 was used to determine significance for all comparisons.

RESULTS

Excellent day-to-day 1RM reliability for each exercise was shown by this protocol. 1RM testing on the two occasions separated by 48 hours showed intra class correlation coefficients of LP r=0.92; LE r= 0.96; BP r=0.94; SP r=0.94; TE r=0.96; LC r=0.94. Additionally, a paired student *t*-test did not show significant differences between the 1-RM tests of any of the exercises.

There were significant differences between SEQA and SEQB in the total number of repetitions completed only for TE, LE and LC exercises (Table 1.1).

TABLE 1.1. Total exercise repetitions for SEQA and SEQB (Mean + SD).

	Bench Press	Shoulder Press	Triceps extension	Leg Press	Leg Extension	Leg Curl
SEQA	19.40 ± 5.62	22.70 ± 9.48	37.50 ± 15.50	42.90 ± 16.00	30.20 ± 12.65	25.10 ± 7.29
SEQB	16.40 ± 10.02	27.30 ± 10.02	51.60 ± 17.07*	37.50 ± 10.82	43.80 ± 11.83*	32.90 ± 7.62*

* Significant difference to SEQA.

The CK concentrations for SEQA and SEQB at different points are shown in Figure 1.2. The CK concentrations were highest at 24 hours following both sessions. When the CK concentrations were compared between SEQA and SEQB, no significant differences were noted at any time point.

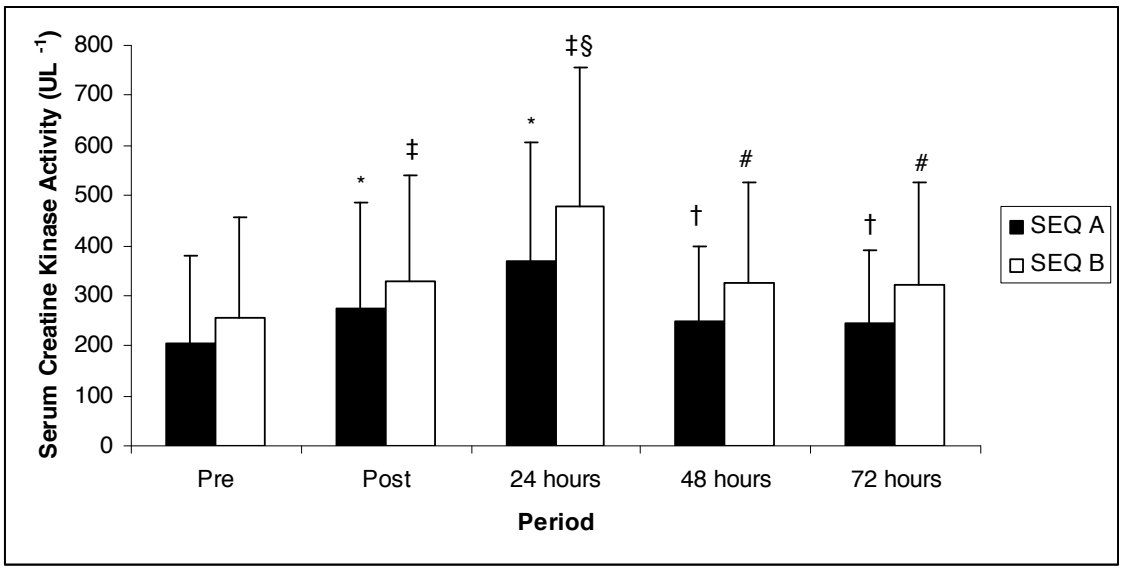

FIGURE 1.2 Serum CK concentrations for SEQA and SEQB at pre, post, 24, 48, and 72 hours.
* Significant difference to pre CK concentration at SEQA.
† Significant difference to CK concentration at SEQA 24 hours after.
‡ Significant difference to pre CK concentration at SEQB.
§ Significant difference to post CK concentration at SEQB.
# Significant difference to CK concentration after at SEQB 24 hours after

The RPE after each exercise was significantly higher in SEQA for TE ($p < 0.04$) and significantly higher in SEQB for LP ($p<0.03$), with no significant differences in BP ($p=0.74$), SP ($p=0.76$), LE ($p=0.74$) and LC ($p= 0.48$).

DISCUSSION

A key finding of the present study was that although some differences exist when the exercise sequence order is changed, those differences could not be explained by muscle damage contradicting our initial hypothesis. This indicates that exercise order did not result in different CK concentrations at multiple time points post training. However, our results also demonstrated that moderately intense sets performed to the point of voluntary exhaustion resulted in elevations in CK concentrations. It occurred in both sequences and did not

show differences between them despite the distinct orders of execution or the total number of repetitions performed in a particular exercise when the total volumes were equal. Similar significant CK elevation at post and 24 hours after both training demonstrates that muscle damage was incurred by both exercise order conditions. These could propose that, in spite of the order of exercises and different number of repetitions for an exercise, both sequences can induce muscle damage. These results are in accordance with previous studies (Clarkson et al 2006, Lee et al 2002, Rodrigues et al 2010) that found elevated blood CK concentration post high intensity strength exercise.

Another important conclusion of this study was that the total number of repetitions for an exercise is directly influenced by the sequence used in a strength training session (Bellezza et al 2009, Dias et al 2010, Farinatti et al 2009, Gentil et al 2007, Sforzo and Touey 1996, Simão et al 2005, Simão et al 2007, Simão et al 2010, Spineti et al 2010, Spreuwenberg et al 2006). In fact, when an upper or lower body exercise for a multi or single-joint was preceded by exercises for the same body part the total number of repetitions in the three sets decreased. This pattern was true for all of the exercises in both SEQA and SEQB. These results corroborate previous studies (Gentil et al 2007, Simão et al 2005, Simão et al 2007) and pointed out that the order of exercise performance should be taken in consideration. Moreover, notwithstanding some small differences in the methodology used, all the studies reviewed (Gentil et al 2007, Simão et al 2005, Simão et al 2007) concluded that the exercise order affects the total number of repetitions. However, no physiological parameters have been considered in order to identify possible mechanisms that would explain this event.

Only three studies that investigated the effect of exercise order and its chronic effects (Dias et al. 2010, Simão et al. 2010, Spineti et al. 2010). Dias et al. (2009) examined the influence of exercise order on strength in untrained young men after 8 weeks of strength training. The small muscle exercises revealed significantly higher strength gains when placed first, demonstrating that the exercise order to small muscle exercises may be particularly important during the initial stages of strength training in untrained young men. On the other hand, Simão et al. (2010) examined the influence of exercise order on strength

and muscle thickness in untrained men after 12 weeks of linear periodized strength training and demonstrated differences in strength and muscle thickness based on exercise order. Spineti et al. (2010) examined the influence of exercise order on strength and muscle volume in untrained men after 12 weeks of nonlinear periodized strength training. The results suggests that different exercise orders during strength training have a significant influence in strength and muscle volume during 12 weeks of nonlinear periodized strength training. In conclusion the three studies, if an exercise is important for individual training goals, it should be performed at the beginning of the training session, whether or not it is a large or a small muscle group exercise. But no study has investigated the influence of order and exercises damage muscle.

Bearing in mind that strength training may induce muscle damage (Clarkson et al 2006, Ferri et al 2006, Jamurtas et al 2006, Lee et al 2002, McBride et al 1998, Zembron-Lacny et al 2008, Rodrigues et al 2010), we have considered the hypothesis of this being one of the mechanisms that could generate differences in total strength production as exercise order changes. Though our results have also suggested that muscle damage has occurred as a consequence of strength training, no differences were found when exercise order changed. Therefore, some other mechanisms, aside from muscle damage, should explain differences in total strength production when exercise order is changed.

The other hypothesis considered refers to the cognitive perception of exercise effort. In the present study, the RPE was higher for TE in SEQA and for LP in SEQB. These exercises were executed in distinct moments of the sequences, TE was the last exercise in SEQA, and the LP was the last exercise for the lower body in SEQB. This suggests that when a small muscle group exercise is performed in the end of the session it presents more difficulties than in the middle. With respect to the decreased number of repetitions, the RPE in exercises performed in the middle of each sequence tended to be greater when compared with the same exercises executed earlier in the sequence. The number of repetitions had also been considered in this study. TE in SEQA had fewer repetitions than in SEQB, probably because in SEQB TE was performed after some rest. In respect to LP in SEQB, when it was executed, two exercises

for the same body part had already been done before and this may have caused fatigue in the lower limbs and evoke a higher RPE. Moreover, in some aspects these results corroborate previous studies (Gentil et al., 2007; Simão, et al., 2005; Simão et al., 2007) as the number of repetitions per exercise performed latter was fewer than when done earlier in the session although the RPE had not changed for different sequences.

CONCLUSION

Although muscle damage appears to be similar as indicated by the lack of differences in CK concentration between the different exercise orders, based on RPE and number of repetitions performed with the different order sequences in the current study, as well as previous studies examining this issue, it appears that exercises that are particularly important to the subject should be placed at the beginning of a training session. This seems to be true for large and small-muscle group exercises, techniques, and genders, levels of training and duration of the exercise for each subject. The study results suggest that whenever one exercise is the last of a sequence performed in a training session, its performance will be negatively affected. This is confirmed whether it is a large or small-muscle group exercise and cannot be explained by differences in muscle damage. The results also indicate that some other mechanisms besides muscle damage and perceived exertion should explain differences in total strength production when exercise order is changed. Therefore, future studies should consider other mechanisms that could be involved.

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3. IS THERE A RELATION BETWEEN MUSCLE DAMAGE AND DOMS AFTER A STRENGTH TRAINING SESSION?

ABSTRACT

This study aimed to investigate if one regular high intensity strength training (ST) session performed to voluntary fatigue could induce muscle damage and similar DOMS responses at multiple time points. For this purpose, ten male subjects performed a session of ST to volitional fatigue and had their CK and DOMS measured before, immediately after and 24, 48 and 72 hours after the exercise session. The CK concentrations were significantly ($p<0.05$) highest at post and 24h following the session in relation to pre exercise. After 48h and 72h of exercise, CK concentrations were lower than at 24 hours after ($p<0.05$). The DOMS values were higher 24h post exercise ($p=0.002$) in relation to pre exercise and decreased after 72h. Significant differences in DOMS were also found between 24h and 72h ($p=0.003$) and 48h and 72h after the exercise ($p=0.005$). These results showed a similar pattern on CK and DOMS values 24 hours after exercise although the statistical analysis did not show a meaningful correlation between these variables. After 72h, the results of CK and DOMS decreased showing significant differences from values at 24h after the exercise. This study suggest that DOMS scale can be used in training routines to appreciate skeletal muscle damage at 24h and the whole body response to ST.

KEYWORDS: strength training, physical activity, DOMS, creatine-kinase.

INTRODUCTION

Strength training (ST) has been studied for several years and is known to promote several benefits such as an increase in muscle hypertrophy, strength, power and local muscular endurance, improving functional abilities as well as quality of life in different types of population. ST has also relevance in sports applications that are related to motor performance improvement, vertical jump, sprint speed, and sport-specific activities associated to others specific techniques (ACSM, 2009). Still, high intensity ST is associated to muscle damage being perceived by the increase in serum creatine kinase (CK) levels after some hours of exercise (Güzel et al., 2007; Zaja et al., 2001).

Serum CK, concentrations may indicate the status of the muscle cell membranes (Clarkson et al., 1992; Jamurtas et al., 2005; Paschalis et al., 2005; Ferri et al., 2006) and can be used as marker of muscle membrane damage or changes in membrane permeability plasma after exercise (Lee et al., 2002; Cheung et al., 2003). It is known that CK activity is elevated for several days following intensive physical activity and that training induces positive adaptive changes that might be observed by a decreased CK levels under a standard load (Clarkson et al., 1986).

Muscle damage can also be present on delayed onset muscle soreness (DOMS). DOMS is a type I muscle injury characterized by muscle pain and other symptoms that occurs 8-10 hours after exercise, with peaks in 12-72 hours after unaccustomed or intense exercise, and mainly after eccentric contractions (Szymanski, 2001; Cheung et al., 2003; Zainuddin et al., 2005; Cleary et al., 2006; Nosaka et al., 2007). The signs and symptoms of DOMS are diffuse pain, discomfort to the touch, stiffness, discomfort, reduced muscle power and peak torque, decrease or loss of muscle strength of the muscle that was exercised, decreased range of motion (ROM), changes in the pattern of recruitment of motor units and decreased neuromuscular function (Szymanski, 2001; Proske, and Morgan, 2001; Cheung et al., 2003; Zainuddin et al., 2005; Cleary et al., 2006, Nosaka et al, 2007).

Although recommended, biochemically control of muscle damage in the exercise's daily programs has many constrains due mainly to the high cost and

unpractical ways to do these tests, and because most of times it requires an invasive technique. Therefore, it became important to identify markers of muscle damage that can be used daily by professionals, in order to measure the individual recovery time after training sessions. The DOMS questionnaire gives an indication of muscle discomfort; however it is a subjective instrument. Thus, we hypothesize that DOMS is correlated with muscular damage evaluated by CK.

Considering that highly fatiguing strength training protocols, such as those that involve moderate intensity sets performed to voluntary exhaustion, may induce significant damage to muscle fibers (Clarkson et al., 1992), we aimed to study whether one regular high intensity strength training session performed to voluntary exhaustion could cause muscle damage and similar DOMS responses at multiple time points. For this purpose, male subjects performed a session of ST to volitional fatigue and had their CK measured before, immediately after and 24, 48 and 72 hours after the exercise and DOMS measured before, immediately after, 24, 48 and 72 hours after the training.

METHODS

Subjects

Ten men (24.50±4.95 yrs; 74.20±7.78 kg; 1.77±0.09 cm; 23.75±0.20 $kg.m^{-2}$, Mean±SD) participated in the study. The following criteria was adopted: a) subjects must have had at least two years of recreational strength training experience; b) subjects couldn't have any medical conditions that might be aggravated by participation in the current study; and c) subjects couldn't be using any nutritional supplements or ergogenic aids. All subjects read and agreed to sign an informed consent document and were asked to avoid any kind of physical activity a week before and during the current study. Experimental procedures were in accordance to the Declaration of Helsinki and the study protocol was approved by the Research Ethics Committee of the Institution.

Delayed Onset Muscle Soreness (DOMS)

The DOMS was measured rated with a visual analog score (VAS 100 mm long) (Price et al., 1983; Clarkson et al., 1992; Cleather et al., 2007) that incorporated a 100-mm line, with zero indicating *no pain* and one hundred representing *extremely painful*. Subjects were asked to mark their whole muscle body perceived soreness on the 100-mm line. Distance from the left edge of the line (0) to the marked point was measured in millimeters, and this value was used for the analysis (Price et al., 1983; Clarkson et al., 1992; Zainudin et al, 2005; Cleather et al., 2007). The muscle soreness values (DOMS) were obtained before, immediately after, 24, 48 and 72 hours post exercise.

Creatine Kinase

To analyze blood CK activity (U/L), capillary blood samples were collected into 80 µL heparinized capillary tubes (Cat nº 955053202 Reflotron®). After the harvest, the blood samples were identified for subsequent use. Serum CK concentrations were determined with 32 µL of blood sample on the Reflotron® Plus – Roche, in which specific reagents (Cat nº 1126695 Reflotron®) were used to analyze the CK concentrations. All blood samples were collected at multiple time points that included: before the bout, immediately after the bout and at the 24^{th}, 48^{th} and 72^{nd} hours after training.

One Repetition Maximum Testing (1RM test)

The 1RM tests were performed on two nonconsecutive days (separated by 48 hours) from which test-retest reliability was calculated (Simão et al., 2007). The 1RM was determined in fewer than five attempts with a rest interval of five minutes between them and 10 minutes between assessments for different exercises. The 1RM tests were performed in a counterbalanced order. The heaviest load achieved on each tests' day was considered the 1RM. To minimize possible errors in the 1RM tests, the following strategies were adopted (Simão et al., 2007): a) all subjects received standard instructions on the general routine of data assessment and proper technique for each exercise before testing; b) the exercise technique of subjects during all testing sessions was monitored and corrected as needed; and c) all subjects received verbal encouragement during testing. The mass of all weight plates and bars used for

measuring 1RM was determined with a precision scale and all machine exercises were performed on Image Sport Machine® (Spain). The same range of motion was used to define a successful repetition for each exercise.

Training session

One week after the 1RM has been determined, subjects performed the exercise protocol. The exercise order was leg press (LP), leg extension (LE), leg curl (LC), bench press (BP), seated machine shoulder press (SP), and triceps extension (TE). The warm-up session consisted of 20 repetitions in the first exercise (LP) at 40% of the 1RM load. A two minutes rest interval was allowed after the warm-up set before subjects performed the sequence. All exercises were performed in three sets to volitional fatigue with 80% of 1RM load with two minutes rest intervals of passive recovery between sets and exercises.

During exercise session, subjects were verbally encouraged to perform all sets to concentric failure. No pause between the eccentric and concentric phases of each repetition was allowed. The total number of repetitions for every set of each exercise was recorded. Strength training session was performed in the morning for all subjects.

Statistical Analyses

Intra-class correlation coefficients (ICC) were used to determine 1RM test-retest reliability. Repeated measures ANOVA were used to compare differences in CK concentrations and DOMS at multiple time points.

Spearman correlation coefficient test (r) was used to evaluate correlation between CK and DOMS responses at multiple time points following the high intensity strength training session. The Statistical Package for the Social Sciences (SPAA version 19.0; Chicago IL) was used for all analyses. The level of significance was defined at $p<0.05$.

RESULTS

An excellent day-to-day 1RM reliability for each exercise was shown by this protocol. The 1RM testing on the two occasions separated by 48 hours showed intra class correlation coefficients of: LP r=0.92; LE r= 0.96; LC r=0.94; BP

r=0.94; SP r=0.94; TE r=0.96. Additionally, the paired student *t*-test did not show any significant differences between the 1-RM tests of all the exercises (Table 2.1).

TABLE 2.1. Number of total exercise repetitions (in three sets at 80% of 1RM) for Leg Press (LP), Leg Extension (LE), Leg Curl (LC), Bench Press (BP) Shoulder Press (SP), Triceps Extensio (TE) (Mean±SD).

Reps	**LP**	**LE**	**LC**	**BP**	**SP**	**TE**
Mean	42.90	30.20	25.10	19.40	22.70	37.50
± SD	±16.00	± 12.65	± 7.29	± 5.62	± 9.48	± 15.50

The CK concentrations and DOMS results at different points are shown in figure 2.1. The CK concentrations were highest at post (272.68 ± 211.64 U/L; Mean ± SD; p=0.002, related to pre) and 24 hours following the session (370.60 ± 235.45 U/L, Mean ± SD; p=0.054, related to pre). After 48h (248.13 ± 149.99 U/L, Mean ± SD); and 72 hours (246.44 ± 145.62, U/L, Mean ± SD) of exercise, CK concentrations were lower than at 24 hours after ($p<0.05$). Concerning to average values of DOMS obtained before training session, values were higher 24 hours post exercise (41.10 ± 29.87, Mean ± SD) and decreased after 72 hours, (12.40 ± 14.23, , Mean ± SD). Significant differences were also found between 24 and 72h (p=0.003) and 48 and 72h (p=0.005). No Significant correlations were found between CK and DOMS.

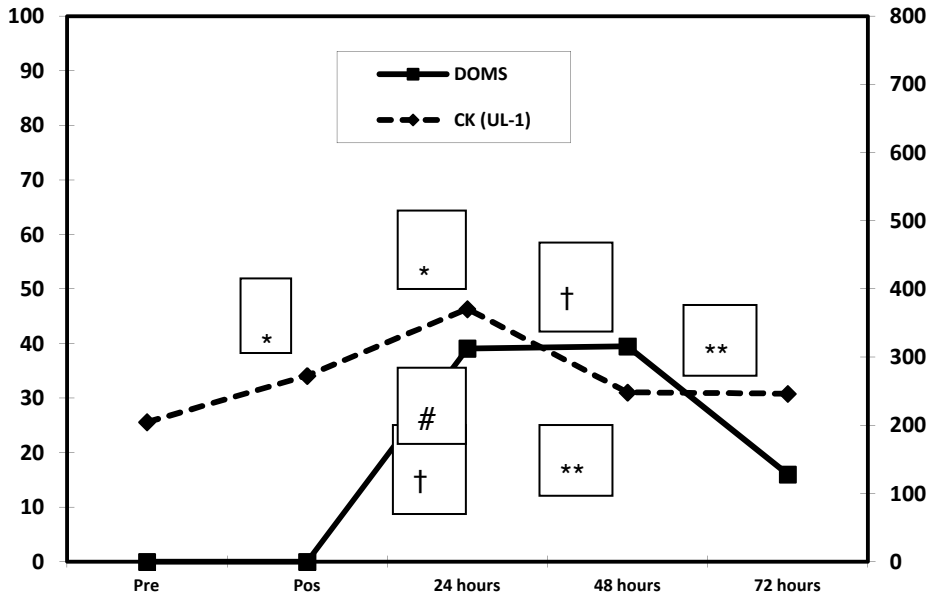

FIGURE 2.1. Serum CK concentrations ($U.L^{-1}$) and DOMS values at pre, post, 24, 48, and 72 hours after exercise.
* Pos and 24 hours - Significant difference to pre CK concentration
(F=18,266; p=0,002) pre and pos; (F=4,925; p=0,054) pre and 24h
** 48 and 72 hours - Significant difference to CK concentration at 24 hours after (F=5,671; p=0,041) 24h and 48h and (F=9,028; p=0,015) 24h and 72h
# 24 hours - Significant difference to DOMS before exercise and 24 hours after (F=18,935; p=0,002).
† 72 hours - Significant difference to DOMS at 24 (F=16,459; p=0,003) and 48 (F=13,432; p=0,005) hours after.

DISCUSSION

It has been suggested in the literature that performing exhausting ST may cause fatigue, protein degradation and muscle cell membrane damage which could generates a release of CK from muscle cells (Aminian-Far et al., 2011; Baird et al., 2012), a macro protein that has been used to estimate cell membrane breakdown (Baird et al., 2012). It seems that the greater the release of CK the greater is the muscle damage (Lee et al., 2002; Cheung et al., 2003). Moreover, muscle damage is usually associated with muscle soreness.

Our hypothesis was that one regular high intensity strength training session performed to voluntary exhaustion could cause muscle damage and that it could be measured through similar changes in CK concentrations and DOMS responses at multiple time points. Our results revealed that CK and DOMS average values were highest 24h after exercise protocol, but while CK became

to decrease afterward, DOMS remained high. So, our results suggest that we may use DOMS scale to evaluate damage associated with strength exercise training, at least 24h after de exercise session. In fact, similar significant CK elevation 24h after training demonstrates that muscle damage was incurred and provoked DOMS. However, although after 48h of training session, CK values start decreasing, DOMS values remained high. It might have happened due to others factors such as immune response induced by exercise which can increase muscle damage (Ziemann, et al., 2013).

These results are in accordance with different previous studies that found elevated blood CK concentration (McBride et al., 1998; Dixon et al., 2006, Clarkson et al., 2006) and DOMS (Lee et al., 2002; Jamurtas et al., 2005; Paschalis et al., 2005), post different high intensity strength training protocols. However these studies used a protocol where the subjects performed the eccentric contractions by using their non-dominant arm, which was not totally based on a protocol used at gym. In the present study, we simulated a general daily protocol used at gym which can partially explain our results and show its importance.

Jamurtas et al. (2005) analyzed two eccentric exercises, one for upper limbs and one for lower limbs in untrained men and showed that there were increases in CK after the two protocols, even with the presence of DOMS and muscle damage in different time recovery and between segments. Paschalis, et al. (2005) found an increase in the CK and the presence of DOMS after a series of maximal voluntary eccentric contractions and at various points after the completion of the protocol. They studied untrained men and used a protocol of two sessions of isokinetic eccentric exercises and had the exercise intensity measured by an isokinetic dynamometer. The volume of the two sessions was considered equal and expressed in Watts. The presence of DOMS and CK increased in the two protocols. Although our study did not test untrained subjects even used an isokinetic dynamometer it showed similar results when related with this one. It is suggested that the intensity of protocol seems to be more important than the training status of the subjects to produce muscle damage associated to DOMS and CK increase; however, untrained subjects tend to generate more DOMS and CK than trained subjects.

According to Jamurtas et al. (2005); Paschalis et al. (2005), Ferri et al. (2006), moderate to high intensity strength training protocols, leading to voluntary exhaustion can induce significant damage in the fibers muscle and we found similar results in our study.

These previous studies have cited generally used protocols with only one exercise, a single muscle group or a circuit format. In none of the studies cited above, the comparison between CK levels and the presence of DOMS were performed after a session of strength training using various skeletal muscle groups, involving both concentric and eccentric muscle actions and exercises for lower and upper-limbs. Nevertheless, studies' evaluating the impact of a strength training protocol, as it is daily performed at the gyms, on blood indicators of muscle injury, and subjective indicators such as subjective DOMS scale (VAS) are unknown.

Our study was based on a protocol used in gymnasium daily basis, and we attempt to show a practical correlation between CK and DOMS. Although the statistical analysis did not present correlation between CK and DOMS, the pattern of the results for CK and DOMS was similar at 24 hours after exercise. Both were higher when comparing to values pre exercise. It is well known that the biochemical control of the muscle damage that results from daily exercise programs implies in a high cost and it is not practical. On the other hand, the DOMS questionnaire is an indicator of muscle discomfort; still it is a subjective instrument. This study tried to establish a correlation between indicators of biochemically muscle damage and a subjective instrument that can be used in a practical way on the daily activities of physical education professionals and technicians, in order to measure the DOMS and the recovery time among the training. In spite of CK levels had demonstrated smaller values by 48 hours and DOMS had decreased just after 48 hours, those findings should be partially explained by our subjects, who were familiar with strength training. It is interesting to observe that as we use a protocol simulating a daily training we could observe muscle damage and DOMS, yet these damage return to rest values faster if compared with protocols cited before which did not simulated an usual training program and studied untrained subjects. We could observe in previous studies that trained subjects can return to rest values faster than

untrained and it allows intensifying the training of these subjects, since the responses of CK and DOMS could also improve with the training.

In this study a protocol simulating a daily training was used, and the use of an eccentric component was not stimulated, once in daily basis, for non-athlete, it is not too much emphasized or needed. Maybe if the protocol had more eccentric than concentric component we could have found a correlation between CK and DOMS, however these values had the same pattern presenting an increase after 24 hours of exercise.

CONCLUSION

The results showed a similar pattern on CK and DOMS values after 24 hours although the statistical analysis did not show a meaningful correlation between them. After 72 hours, the results of CK and DOMS decreased showing significant differences as compared to the values after 24 hours. This can be explained by the fact that the subjects where more familiar with the strength training. One might consider that for trained people or athletes an eccentric protocol should be recommended in addition to high intensity protocols in order to generate greater muscle damage. These results suggest that DOMS scale can be used as an important ratio to measure muscle damage associated with strength exercise training at least 24h after de exercise session.

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4. MODIFICATION OF LYMPHOCYTE DNA DAMAGE AFTER A SESSION OF STRENGTH TRAINING WITH SUBMAXIMAL REPETITIONS

ABSTRACT

This study checked whether a session of strength training (ST) with maximal repetitions induces lymphocytes DNA damage immediately after, 24h, 48h and 72h of completing a strength exercise protocol and whether these changes follow a similar pattern of those found in skeletal muscle assessed by CK. For this purpose, ten healthy male subjects performed a session of ST to volitional fatigue and had their capillary blood samples measured before, immediately after and 24h, 48h and 72h after the exercise for CK and DNA lymphocytes analyze. The CK concentration was highest ($p<0.05$) at pos and 24h following the session when compared to pre exercise and decreased ($p<0.05$) at 48h and 72h post exercise compared to 24h ($p<0.05$). DNA results revealed significant differences in damage measured through DNA strand breaks ($p<0.05$) between 24 and 48 hours after completion of the protocol, and also revealed significant differences in DNA FPG sites between 24h and 48h ($p<0.05$) and between 24h and 72h after exercise ($p<0.05$). Our results showed that up to 24h after a ST protocol, there is a similar trend between CK and DNA damage increase, showing that a ST session can generate damage at muscle membrane as well as DNA in lymphocytes causing and/or caused by oxidative stress increase. According to the results of this study, these damages were maintained until 24h after the completion of the protocol, returning to below resting values after 48h of the protocol completion.

KEYWORDS: strength training, physical activity, creatine kinase, DNA in lymphocytes, muscle damage, stress oxidative.

INTRODUCTION

Strength training (ST) has been recommended as part of a physical training program and is being built in conditioning programs for improving the health and quality of life in various types of population (ACSM, 2009). ST induces several benefits (ACSM, 2002, 2009; Komi, 2003; Carroll et al., 2001) but when performed at high intensity can cause muscle damage (Clarkson et al., 2006). These damages may be of metabolic, mechanical, or inflammatory causes. Muscle damage may be also associated with vascular injury which may induce an increased immune response that will exacerbate muscle damage hours (days) after the workout. In the aerobic training, these immunological changes are accompanied by an increase in oxidative damage in various cells (Mastaloudis et al., 2001; Voollard et al, 2005). However, with regard to ST, a few studies of this nature exist and have used protocols with high numbers of repetitions of the same exercises (Brown et al., 1997).

However, since the ST induces damage to muscle membrane integrity (Nosaka and Clarkson, 1995; Nikolaidis et al., 2008) that allow the release of cytokines that lead to an increased immune system response, causing an increase in oxidative stress, it seemed appropriate to check how far the muscle damage caused by a high intensity ST protocol can cause oxidative damage to DNA in lymphocytes blood cells, resembling the effects observed skeletal muscle. So, the aim of this study was to check if a session of strength training with sub-maximal repetitions for upper and lower limbs in subjects familiarized with strength training induces DNA lymphocytes damage immediately after, 24, 48, 72 hours of completing the exercise protocol and whether these changes follow a similar pattern of change in muscle membrane damage assessed by CK.

METHODS

Subjects

Ten men (24.50 ± 4.95 yrs; 74.20 ± 7.78 kg; 1.77± 0.09 cm; 23.75 ± 0.20 $kg.m^{-2}$, Mean± SD) participated in the study. The following criteria was adopted: a) subjects must have had at least two years of recreational strength training

experience; b) subjects couldn't have any medical conditions that might be aggravated by participation in the current study; and c) subjects couldn't be using any nutritional supplements or ergogenic aids. All subjects read and agreed to sign an informed consent document and were asked to avoid any kind of physical activity a week before and during the current study. Experimental procedures were in accordance to the Declaration of Helsinki and the study protocol was approved by the Research Ethics Committee of the Institution.

One Repetition Maximum Testing (1RM test)

The 1RM tests were performed on two nonconsecutive days (separated by 48 hours) from which test-retest reliability was calculated (Simão et al., 2007). The 1RM was determined in fewer than five attempts with a rest interval of five minutes between them and 10 minutes between assessments for different exercises. The 1RM test order was leg press (LP) 45^0, leg extension (LE), leg curl (LC), bench press (BP), seated machine shoulder press (SP), and triceps extension (TE). The heaviest load achieved on each tests' day was considered the 1RM. To minimize possible errors in the 1RM tests, the following strategies were adopted (Simão et al., 2007): a) all subjects received standard instructions on the general routine of data assessment and proper technique for each exercise before testing; b) the exercise technique of subjects during all testing sessions was monitored and corrected as needed; and c) all subjects received verbal encouragement during testing. No pause between the eccentric and concentric phases of each repetition was allowed. For a repetition be accepted, the complete range of motion of the exercise had to be conducted. The mass of all weight plates and bars used for measuring 1RM was determined with a precision scale and all machine exercises were performed on Image Sport Machine® (Spain). The same range of motion was used to define a successful repetition for each exercise.

Training Session

One week after the 1RM has been determined, subjects performed the exercise protocol. The exercise order was leg press (LP), leg extension (LE), leg curl

(LC), bench press (BP), seated machine shoulder press (SP), and triceps extension (TE). Warm-up session consisted of 20 repetitions in the first exercise (LP) at 40% of the 1RM load. A two minutes rest interval was allowed after the warm-up set before subjects performed the sequence. All exercises were performed in three sets to volitional fatigue with 80% of 1RM load with two minutes rest intervals of passive recovery between sets and exercises.

During exercise session, subjects were verbally encouraged to perform all sets to concentric failure. No pause between the eccentric and concentric phases of each repetition was allowed. The definitions of complete range of motion to characterize a complete repetition of each exercise were the same as described in the 1RM test. The total number of repetitions for every set of each exercise was recorded for later analysis and comparisons of aggression muscle (CK), oxidative stress and DNA damage in lymphocytes. Strength training session was performed in the morning between 9:00 and 12:00 am for all subjects.

Blood Collection

Blood was collected before, immediately after the bout and at 24, 48 and 72 hours after completion of the exercise protocol between the hours of 9 to 12pm. Capillary blood samples was obtained from each subject by puncture in the finger, proceeding to the collection of 70µL for comet assay test and 80 µL for CK analysis. After collection, the samples were identified and preserved in microtubes in a container at 4°C until be analyzed.

Creatine Kinase

To analyze blood CK activity (U/L), capillary blood samples were collected into 80 µL heparinized capillary tubes (Cat nº 955053202 Reflotron®). After the harvest, the blood samples were identified for subsequent use. Serum CK concentrations were determined with 32 µL of blood sample on the Reflotron® Plus – Roche, in which specific reagents (Cat nº 1126695 Reflotron®) were used to analyze the CK concentrations. All blood samples were collected at multiple time points that included: before the bout, immediately after the bout and at the 24^{th}, 48^{th} and 72^{nd} hours after training.

DNA Damage

The disruption of DNA strands were evaluated using the comet assay technique or single-cell electrophoresis gel following the method of Collins (2004).

After blood collection the lymphocytes isolation have been proceeded. The blood was diluted with phosphate buffer solution (PBS) (1x) in 1.5 mL Eppendorf microtubes, homogenizing with careful and without stirring. Then, with the micropipette at the bottom of the microtube, 100mL of lymphocyte isolation solution (RAFER - Cora) was added, being careful not to mix the different phases. The microtubes were taken to the centrifuge at 4°C for 5 minutes at 200xg. After centrifugation, 100mL of lymphocyte layer (layer between the lymphocyte isolation solution and the PBS) were taken to another microtube.

Lymphocytes were washed with 1 mL of PBS and homogenized well and without stir. A further centrifugation was carried out at 200xg at 4°C and supernatant was discharged. 280µL of low melting point (LMP) agarose was added to the pellete. Once gently and without stirring homogenized, two drops of sample (2x70µL) were placed in a slide, and immediately covered with two lamellas of 18x18mm for later solidification in a refrigerator during 5 minutes.

The sipes were then removed from the blades and placed in a vertically container with 1ml of Triton X-100 in 100 ml of lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, pH 10, 4°C) and kept in dark for 1h. The slides were then washed in three changes of enzyme reaction buffer (40 mM HEPES, 0.1 MKCl, 0.5 mM EDTA, 0.2 mg / ml BSA, pH 8.0 with KOH, 4 °C) in a staining for 5 min each time. After removing the blades the last wash, the liquid excess was removed with blotting paper.

Two blades were made per person, one to measure basal DNA breaks and the other to identify DNA oxidative damage by incubation with enzyme formamidopyrimidine DNA glycosylase (FPG). This enzyme is lesion´s specific that detects this type of damage and creates a break during incubation which increases its sensitivity and its specificity. Fifty microliters of enzyme solution (or buffer alone as a control) was put on each gel and covered with a 22x22 mm

coverslip. The slides were placed in a humid box (to prevent dehydration) and incubated at 37°C for 30 min for the activity of FPG. After this incubation, the slides were gently removed and placed (with the exception of the lamellae) in platforms in an electrophoresis tank, forming one or two complete lines, and immersed in a electrophoresis solution (0.3 M NaOH and 1 mM EDTA) for 40 min (4°C). Then, electrophoresis was carried out for 30 min at 25 V (constant voltage adjustment). Thereafter, the slides were washed three times with neutralization buffer (0.4 M Tris, pH to 7.5 with concentrated HCl) in a staining for 5 minutes at 4 °C. The slides were then stored at room temperature. The slides were stained with ethidium bromide (2 µg/ml) immediately prior to its display on a fluorescence CARL ZEISS microscope. The tail intensity was measured in each cell by the visual method classifying them into five classes which represent different degrees of DNA damage, ranging from 0 (no cell migration observable and therefore no damage) to 4 (severe observable cells migration and intense damage). In each drop, 100 cells were observed, since each blade had two drops, the average of both drops was calculated with a score ranging from 0 (no damage) to 400 (completely damaged; Collins 2004). The classification of comet assay was performed blindly by an experienced researcher in the field. After at least a month, 10% of the slides were viewed again for the test-retest reliability.

Statistical Analyses

Intra-class correlation coefficients (ICC) were used to determine 1RM and comet assay test-retest reliability.

Exploratory analysis revealed a normal distribution of data. Repeated measures ANOVA were used to compare differences between the values of DNA strand breaks (no enzyme) and the DNA FPG (oxidative stress).

For the identification of differences between variables proceeded to the realization of Turkey Post Hoc Test.

The Statistical Package for the Social Sciences (SPSS version 16; Chicago IL) was used for all analyses. The level of significance was defined at $p=0.05$.

RESULTS

Excellent day-to-day 1RM reliability for each exercise was shown by this protocol. 1RM testing on the two occasions separated by 48 hours showed intra class correlation coefficients of: LP r=0.92; LE r= 0.96; LC r=0.94; BP r=0.94; SP r=0.94; TE r=0.96. Additionally, the paired student *t*-test did not show any significant differences between the 1-RM tests of all the exercises. The comet assay reliability was significant (r = .94).

The CK concentration was highest at 24 hours following the session figure 3.1.

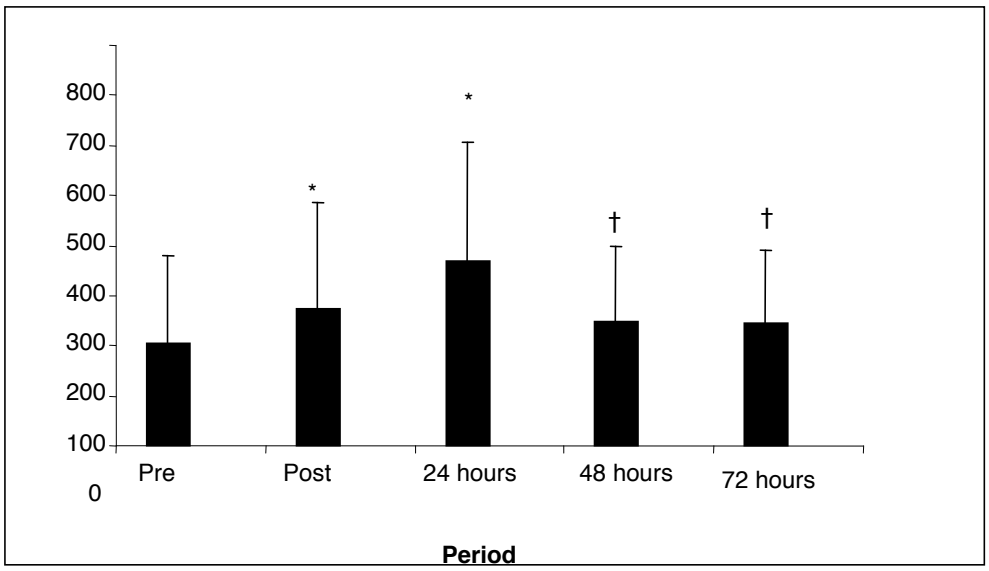

Figure 3.1. Serum Creatine Kinase Pre, Post, 24, 48, 72 hours after the completion of protocol.

The CK concentrations were highest at 24 hours following the session.
* Significant difference to pre CK concentration.
† Significant difference to CK concentration 24 hours after.

Based on the results it was observed an increase in values basal damage of DNA2 (DNA strand breaks) immediately after the execution of the protocol (mean ± SD, 38.56 ± 15.79) until 24 hours after the completion of the exercise (mean ± SD, 47.13 ± 26.54) at 48 and 72 hours after the completion of the protocol, the values were lower than before execution thereof (mean ± SD, 20 44 ± 4.58 and 26.25 ± 6.21), respectively (Figure 3.2).

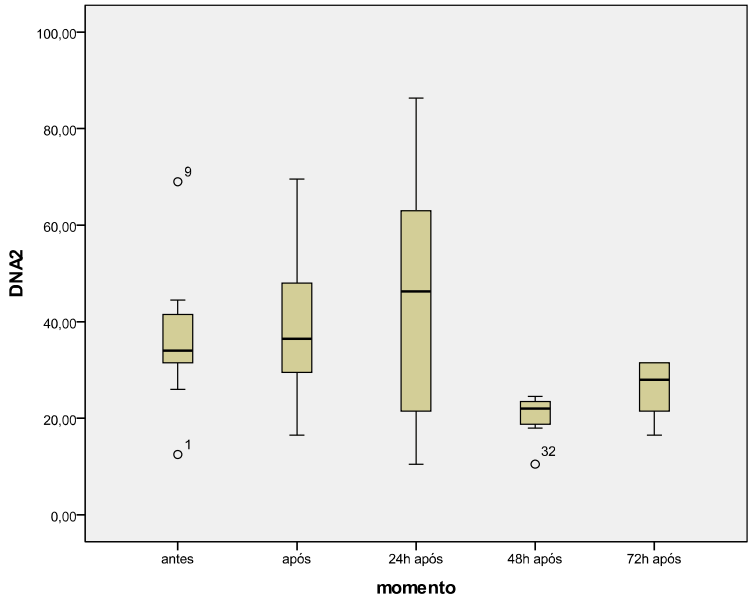

Figure 3.2. DNA Strand Breaks (DNA2) Results

In relation to DNA damage by oxidative stress (DNA FPGSites) values obtained prior to the protocol were (56.72 ± 15.70 mean ± SD) immediately after and 24 hours after completion of the protocol the values increased to (60.93 ± 36.50 and 72.45 ± 36.36, mean ± SD) respectively and after 48 and 72 hours the average and standard deviation decreased to 31.50 ± 7.87 and 36.75 ± 13.70, respectively (Figure 3.3).

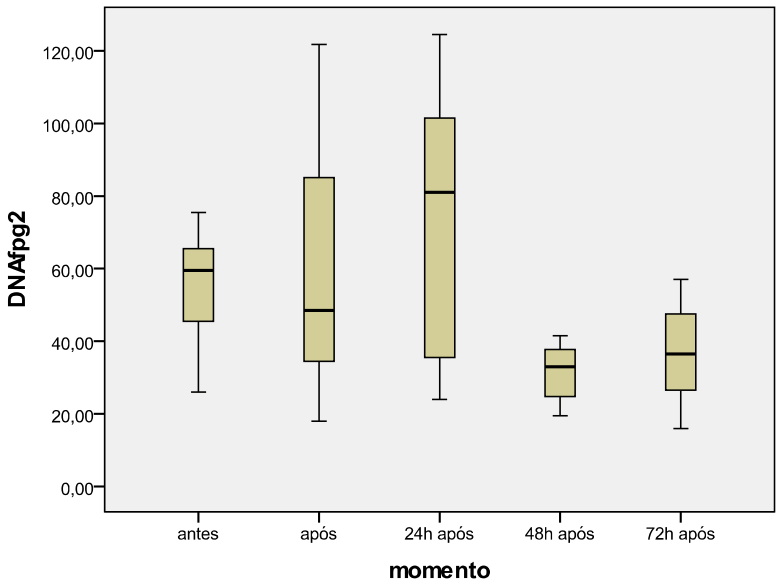

Figure 3.3. DNA FPG Sites (DNAfpg2) Results

The ANOVA revealed differences between the moments after the protocol execution The post hoc revealed differences in the damage of DNA strand breaks (Mean ± SD, 24.86 ± 7.56, p = 0.017) between 24 and 48 hours after completion of the protocol, and also revealed differences in DNA FPG sites between 24 and 48h (mean ± SD, 40.95 ± 12.46, p = 0.017) and between 24 and 72 hours after exercise (mean ± SD, 35.70 ± 12.46, p = 0.049).

DISCUSSION

This study aimed to determine whether a session of ST with sub-maximal repetitions induces DNA damage in lymphocytes at various times after the completion of an exercise protocol, as well as whether these changes follow a pattern of change similar to the damage of muscle membrane assessed by CK.

Our results show that up to 24 hours after a ST protocol for the lower and upper limbs, values of DNA strand breaks, DNA FPG sites as well as CK values were increased relative to the resting values. It seems that there is a trend between increasing CK and DNA, after conducting an exercise session, showing that a ST session can generate damage at muscle membrane as well as in blood cells caused by oxidative stress. Thus, one can already consider the presence of both, muscle membrane damage and DNA oxidative damage in lymphocytes after a sub-maximal ST session. According to the results of this study, these damages are maintained until 24 hours after the completion of the protocol, returning to below resting values 48 hours after. This is supported by exercise prescriptions, since the ST sessions occurs mostly every other day. However, has the differences were not significant, we can only assume that this session induced some damage, eventually the one necessary to promote organism adaptations and improvement. In fact, acute exercise is a stress condition that through a metabolic and mechanical load can induce changes and functional increase. This study was performed in young healthy subjects who can also explain the low damage observed after the ST session. Considering that strength training can improve muscle function and hypertrophy, particularly important in older frail people, it could be of major importance to repeat this study with elderly subjects.

It can also be considered the possibility of separating the training according to muscle groups, to check possible differences in the susceptibility to damage and to figure out the related differences in recovery time.

CONCLUSION

This study was important to clarify that a sub-maximal ST for upper and lower limbs generates lymphocyte oxidative stress damage in DNA and muscle damage up to 24 hours after completion of the protocol and this should be considered in the practice of prescribing exercise at gyms, thus avoiding possible injuries and complications training.

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5. GENERAL DISCUSSION

General Discussion

The ST has been recommended as part of a physical training program and, undoubtedly, it is considered an effective method for increasing strength, power, endurance and muscle hypertrophy (ACSM, 2002, 2009; Carroll et al. 2001; Komi, 2003). However, the benefits attributed to ST depend on the number of variables, including the number of sets, number of repetitions, load intensity, order of exercises, and resting periods between sets, among others (ACSM, 2009). Thus, a proper handling of variables is required to generate different responses and adaptations to achieve the purposes desired by trained persons.

This thesis aimed to study the influence of exercise order in the number of repetitions at 80% of 1RM to volitional fatigue, and searched for possible underling mechanisms that could explain those differences.

One of the possible underling mechanism proposed to explain the influence of exercise order in the number of repetitions, was that muscle damage should be different. Indeed, it was hypothesized that the higher number of repetitions by bigger skeletal muscle groups (sequence proposed by ACSM, 2009) would induce more damage and so, CK concentrations would be higher. Moreover, once exercises were performed to volitional fatigue it would expect that higher perception of effort would be related with higher number of repetitions and possibly with greater muscle damage. These hypotheses were explored in the first study where we studied the number of repetition, effort exercise perception and muscle damage after two different sequences of strength training with sub-maximal repetition to volitional fatigue. We observed differences in the number of repetitions in SEQB for TE (51.60 vs. 37.50), for LC (32.90 vs. 25.10) and for LE (43.80 vs. 30.20) when compared to SEQA, demonstrating that the order affects the number of repetitions. These results suggest that when working with normal or disable persons it is important to identify each one skeletal muscle unbalance giving to those skeletal muscles priority in the training session.

Regarding skeletal muscle damage, our results revealed a significant increase immediately and 24h after the exercise session. However, no difference was observed with the exercise sequence, suggesting that muscle damage should not be responsible for differences in the number of repetitions. Other

mechanisms should be involved, possibly related to the effort perception. In fact, our results revealed an increased perception of effort after the triceps movement in the first sequence (SEQA) and for leg press in the second sequence (SEQB). It worth to remember that in the first sequence triceps was the last exercise of the session, so fatigue, either others factors such as physiological and/or psychological, should have influenced the ability to perform correctly the technique of the exercise so the exercise had to be interrupted. The same could justify the results observed in the leg press, once in the SEQB this was the last exercise for lower-body. In relation to order we were in line with previous studies (Simão et al. 2005; Simão et al. 2007; Rodrigues, et al., 2010, Spinet at al., 2010) and this study came to show that CK will have a similar response even when the order changes. It should be considered when prescribing exercises in day-to-day gym, since the manipulation of variables takes distinct subjective and physiological responses.

Once the proposed strength training session was able to induce muscle damage it becomes meaningful to search for related signs of pain, namely delayed onset muscle soreness (DOMS). So, in the second study we have looked for the relationship between subjective indicators of DOMS and the presence of muscle damage on performance of a sub-maximal protocol of strength training. An increase in CK and DOMS was observed 24 hours after the completion of the protocol thus suggesting that the scale of DOMS can be used after a training session referring to the presence of muscle damage at this point which is corroborated with previous studies (Clarkson et al. 1992; Proske et. al, 1998). Although CK had decreased 48h after exercise, the DOMS remained elevated, and this could suggest that muscle damage could be related to other systemic mechanisms or nervous. Our results demonstrated that we can use a perceived effort scale as a proxy to gauge muscle damage at least during 24h after exercise. The use of DOMS scale seems to be a very useful, practical and feasible method to control exercise load intensity and rest between exercise training sessions. Having in mind that people that look forward to train in gym differ in their genetic potential, gender, age, training and nutrition status that could influence the recovery capacity, we may recommend

the use of DOMS scale to adjust individual sessions frequency, providing a better adaptation to training.

Another physiological mechanism that could be involved in delayed muscle damage is related with the possible increase in immunological response to exercise, frequently associated with oxidative stress damage (Neubauer et al., 2008; Ziemann, et al., 2013). So, in the third study we examined whether a session of strength training with sub-maximal repetitions induced oxidative damage to DNA in lymphocytes and how this damage was related to muscle damage. The DNA Strand Breaks and DNA FPG rose immediately after the completion of the exercise protocol and remained elevated for up to 24 hours after the completion of strength protocol suggesting that ST can cause muscle damage with systemic implications. The variations of CK and lymphocytes DNA damage followed a similar pattern, which suggest that for these subjects the recovery mechanisms were clearly manifested 48 hours after completion of the ST protocol. These data also suggest that muscle damage caused by the completion of a high intensity ST protocol can cause local and systemic oxidative stress causing DNA damage to lymphocytes.

Altogether, our results suggest the importance of at least 48 hours of rest between the executions of two sessions of strength training. This study also suggest that more mechanisms related with DOMS in the ST should be explored once we could not explain the elevated values observed 48 hours after the training session.

6. GENERAL CONCLUSIONS

General Conclusions

We may conclude that in general conducting a training session of high intensity strength for subjects familiar with strength training can cause muscle damage and lymphocyte DNA damage and that some of such damage can be estimated indirectly by the DOMS scale. We can also conclude that the order in which the exercises are performed in the series may affect results and it seems that the exercises that are particularly important to the objectives of the individuals who are being trained should be placed at the beginning of a training session. The study results suggest that whenever one exercise is the last of a sequence performed in a training session, its performance will be negatively affected. The results also indicate that some other mechanisms besides muscle damage and perceived exertion should explain differences in total strength production when exercise order is changed. Therefore, future studies should consider other mechanisms that could be involved.

Future Directions:

- Studies using different populations such as elderly, for example, seem to be interesting in order to verify if the answers follow the same trend.
- Studies using other parameters as inflammation indicators, electromyography and 1RM test in 24h, 48h and 72h after the protocol are suggested.
- Studies on chronic effects of the exercises order on muscle hypertrophy and underlying physiological mechanisms are welcome.

This study seems to be very useful and important in the daily basis gymnasiums activities.

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