

ALTERATIONS IN PEAK TORQUE OCCUR WITHOUT HEMATOLOGICAL CHANGES AFTER MUSCLE FATIGUE



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AS ALTERAÇÕES NO PICO DE TORQUE OCORREM SEM MODIFICAÇÕES EM PARÂMETROS HEMATOLÓGICOS APÓS FADIGA MUSCULAR

LOS CAMBIOS EN EL PICO DE PAR OCURREN SIN MODIFICACIONES EN PARÁMETROS HEMATOLÓGICOS DESPUÉS DE FATIGA MUSCULAR

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ABSTRACT

Introduction: Muscular fatigue during voluntary muscle contractions is a complex and multifactorial phenomenon associated with central changes and adaptations of the neuromuscular system. **Objective:** The aim of this study was to analyze the changes in both peak torque and hematological parameters in active and inactive healthy subjects following a muscular fatigue protocol. **Methods:** Twenty-one healthy adult males performed a muscle fatigue protocol (10 sets, 10 repetitions of knee extension at 75% of the maximal isometric torque (MIT), 120^{sec}-1 and 40 seconds of rest). Hematocrit (Ht), red blood cells (RBC) and leukocytes (L) analysis were evaluated before (B) and after (A) a fatigue test. **Results:** The inactive group presented impairment only in concentric muscular action of -41±4% in relation to the active (-23±3%) group. No differences were found in hematological parameters in inactive group (Ht, B: 45.00±0.01 vs. A: 47.00±0.01; RBC, B: 442±40 vs. A: 487±41; L, B: 7,565±2,878 vs. A: 8,015±4,224) and the active group (Ht, B: 45.00±0.63 vs. A: 47.00±0.01; RBC, B: 477±30 vs. A: 559±37; L, B: 6,418±3,557 vs. A: 6,632±4,460). **Conclusion:** Differences were found only in concentric actions between groups. Additionally, there were no relationships found in hematological parameters between groups.

Keywords: muscle fatigue; muscle, skeletal; muscle strength; physiology.

RESUMO

Introdução: A fadiga muscular durante as contrações musculares voluntárias é um fenômeno complexo e multifatorial associado a alterações centrais e adaptações do sistema neuromuscular. **Objetivo:** O objetivo deste estudo é analisar as alterações do pico de torque e dos parâmetros hematológicos em indivíduos saudáveis ativos e inativos depois de realizar um protocolo de fadiga muscular. **Métodos:** Vinte e um adultos saudáveis do sexo masculino realizaram um protocolo de fadiga muscular (10 séries, 10 repetições de extensão do joelho a 75% do torque isométrico máximo (TIM) a 120^{sec}-1 e 40 segundos de repouso). Hematócrito (Ht), glóbulos vermelhos (GV) e leucócitos (L) foram analisados antes (A) e depois (D) do teste de fadiga. **Resultados:** O grupo inativo apresentou deterioração apenas na ação muscular concêntrica (-41 ± 4%) com relação ao grupo ativo (-23 ± 3%). Não foram encontradas diferenças nos parâmetros hematológicos no grupo inativo (Ht, A: 45,00 ± 0,01 vs. D: 47,00 ± 0,01; GV, A: 442 ± 40 vs. D: 487 ± 41; L, A: 7.565 ± 2.878 vs. D: 8.015 ± 4.224) e no grupo ativo (Ht, A: 45,00 ± 0,63 vs. D: 47,00 ± 0,01; GV, A: 477 ± 30 vs. D: 559 ± 37; L, A: 6.418 ± 3.557 vs. D: 6.632 ± 4.460). **Conclusão:** Foram encontradas diferenças apenas em ações concêntricas entre os grupos. Além disso, não foram encontradas relações quanto aos parâmetros hematológicos entre os grupos.

Descritores: fadiga muscular; músculo esquelético; força muscular; fisiologia.

RESUMEN

Introducción: La fatiga muscular durante las contracciones musculares voluntarias es un fenómeno complejo y multifactorial asociado con cambios centrales y adaptaciones del sistema neuromuscular. **Objetivo:** El objetivo de este estudio es analizar los cambios del par máximo y los parámetros hematológicos en sujetos sanos activos e inactivos después de un protocolo de fatiga muscular. **Métodos:** Veintiún adultos sanos del sexo masculino realizaron un protocolo de fatiga muscular (10 series, 10 repeticiones de extensión de la rodilla en el 75% del par isométrico máximo (PIM) a 120^{sec}-1 y 40 segundos de descanso). Hematocrito (Ht), glóbulos rojos (GR) y leucocitos (L) fueron evaluados antes (A) y después (D) de la prueba de fatiga. **Resultados:** El grupo inactivo presentó deterioro sólo en la acción muscular concéntrica (-41 ± 4%) con respecto al grupo activo (-23 ± 3%). No se encontraron diferencias en los parámetros hematológicos en el grupo inactivo (Ht, A: 45,00 ± 0,01 vs. D: 47,00 ± 0,01; GR, A: 442 ± 40 vs. D: 487 ± 41; L, A: 7.565 ± 2.878 vs. D: 8.015 ± 4.224) y en el grupo activo (Ht, A: 45,00 ± 0,63 vs. A: 47,00 ± 0,01; GR, A: 477 ± 30 vs. D: 559 ± 37; L, A: 6.418 ± 3.557 vs. D: 6.632 ± 4.460). **Conclusión:** Se encontraron diferencias sólo en las acciones concéntricas entre los grupos. Además, no hubo relaciones con respecto a los parámetros hematológicos entre los grupos.

Descritores: fatiga muscular; músculo esquelético; fuerza muscular; fisiología.

INTRODUCTION

Conceptually, muscle fatigue corresponds to an inability to maintain skeletal muscle strength levels for a certain period of time¹. Studies² investigating muscle fatigue have been published that consider the multiple facets of the associated fatigue mechanisms, with a view to understanding the fatigue process. Some of the mechanisms involved in the fatigue process², include changes in electromyography³, mechanical alterations⁴, changes in blood biochemistry⁵ have received much attention. However, research related to hematological change is still inconclusive⁶. Some studies⁷ have outlined this research limitation during studies investigating biochemical associations and fatigue profiles following exercise.

In addition, fatigue profiles of individuals with different levels of fitness and levels of physical activity have received little attention in the scientific community. Knowledge about the relationship between physical activity as a risk factor for chronic degenerative disease is already well established in the literature⁸. The level and intensity of physical activity has been recognized as a component that influences muscle performance^{9,10} by promoting force development and endurance. However, information relating to the mechanisms influencing performance when this variable is considered remains inconclusive.

Therefore, considering the lack of information in this area, especially the relationship between the level of physical activity and changes in hematological parameters, the objective of this study, was to investigate any changes in force development following a fatigue protocol. A secondary objective was to consider muscle action and changes in hematological parameters of active and inactive individuals undergoing a specific muscle fatigue protocol.

MATERIALS AND METHODS

Following approval by the Research Ethics Committee of the São Judas Tadeu University (number 786.06), twenty male adults aged ≥ 18 years and physically independent signed an Informed Consent form to participate voluntarily in this study. The International Physical Activity Questionnaire (IPAQ) - short version was used to estimate the level of physical activity conform previously publication of our group¹⁰. Questions were based on the level of physical activity performed weekly. The questions explored frequency and duration of exercise, including moderate walking, vigorous exercise and time spent sitting down. Subjects were considered active if they reached or exceeded 150 minutes of physical activity per week. Subjects who did not reach 150 minutes per week were classified as inactive. Classifications were based on the guidelines outlined by the World Health Organization. Subjects with diabetes mellitus, current smokers, individuals with musculoskeletal injuries and diagnosed clinical case pathologies and/or cardiovascular changes confirmed by medical evaluation, were excluded from the study.

Anthropometric measurements

Anthropometric assessment was performed following the model previously outlined by our group^{11,12}. Briefly, height was measured with a Cardiomed stadiometer (WCS model), with a 115/120 cm range. The measurement was performed with the cursor placed at a 90° angle with the scale in the upright position, and feet placed together in contact with the stadiometer. Subjects were instructed to hold their breath and keep the head parallel to the ground. Weight was measured with a calibrated electronic scale (Filizola - Personal Line Model 150), with a 100 grams range, and maximum capacity of 150 kg. Body mass index (BMI) was calculated and recorded from the equation weight/height¹.

Fatigue induction protocol

The completion of the dynamic fatigue protocol followed the model used of our group¹⁰. Briefly, all subjects attended the Laboratory on four

occasions to perform an Isokinetic evaluation protocol using a Biodex System 3 (Biodex, Inc., Shirley, NY). On the first day, the subjects signed consent forms and completed the IPAQ. On the second day, anthropometric data (height, and weight) was evaluated. On the third day, the subjects were placed on the Isokinetic Dynamometer in a comfortable, upright, seated position for familiarization procedures. Straps were used to fix the thigh, pelvis, and trunk to prevent extraneous body movement. The axis of the dynamometer was aligned with the axis of right knee joint rotation. Arms were placed across the chest with hands grasping the straps. All subjects performed eight repetitions of sub maximum concentric/eccentric knee extension/flexion at 120°⁵¹. The knee joint range of motion was 90 to 10° of knee flexion. The calibration of the Isokinetic Dynamometer was performed according to the specifications of the manufacturer before each test, and finally, on the fourth day the fatigue protocol was performed. All testing and experimental procedures were performed at the same time of day to exclude diurnal variation. The fatigue protocols were performed at an intensity of 70% of maximum isometric contraction, which was determined on the third day.

Evaluation of hematological indicators

To establish hematological changes, blood was collected with a 5 ml syringe using methodologies outlined previously¹⁰. Subsequently, the blood was transferred into tubes containing EDTA and kept in motion to avoid coagulation. The hematocrits were analyzed with the microhematocrit technique, which consists of collecting blood in a microcapillary tube of up to $\frac{3}{4}$ of its capacity, sealing one end with plasticine, then centrifuging for five minutes at 10,000 rpm in a microcentrifuge. The reading was recorded from a table for hematocrit, considering between 40 and 50% of red blood cells (RBC) as normal values. For the RBC count, 20 ml of blood was diluted in 5 ml of citrate formaldehyde, with subsequent counting in a Neubauer chamber. For the establishment of leukocyte count, the same amount of blood was diluted in 4000I Turk, with subsequent counting in a Neubauer chamber. Cells were counted in a manual counter (model SYSMEX-SF- 3000) in the specific areas for each cell type with appropriate calculations of dilutions performed. A slide containing a blood smear was used to verify the morphology and the usefulness of cells. Smears were made by dragging a drop of blood by contact with another slide, and the material was placed to dry naturally. After complete drying, the slides were subjected to the Fast Panoptic method, with a methanol bath for five seconds for fixation, eosin for five seconds, and ten seconds in Methylene Blue for coloring. Then, slides were washed with deionized water and observed under an optical microscope.

Statistical analysis

The analyses were performed using the SPSS for Windows (version 12.0; SPSS, Chicago, IL, USA). The D'Agostino-Pearson test was used for data normalization. For comparison group analysis was used the Student's t test or two-way ANOVA for repeated measures, followed by a post- hoc Bonferroni test as needed. Data are expressed as mean and standard error of the mean, and the significance level was set at $p < 0.05$.

RESULTS

After analyzing the IPAQ questionnaire, seminal time spent on physical activity of the inactive group corresponded to 121 ± 36 minutes, which was significantly lower ($p = 0.001$) than the active group (657 ± 267 min). In relation to anthropometric parameters no differences were found between groups, as shown in Table 1.

After application of the fatigue-inducing protocol, significant differences ($p < 0.01$) in peak torque were found for both groups after the second set, both for the concentric (Figure 1A) and eccentric muscle action (Figure 1B). Significant differences ($p < 0.001$) in the force-repetition

curve slope were found only in the eccentric peak torque between groups (active: -6.30 ± 0.61 , inactive: -9.55 ± 12.29 ; Nm/series). No difference was found in the eccentric peak torque between active (-13.24 ± 0.62 Nm/series) and inactive groups (-14.28 ± 0.77 Nm/series).

Additionally, a significant percentage reduction ($p < 0.001$) was found after ten sets at torque maintenance of both groups in both the concentric (active: -23 ± 2 , inactive: -41 ± 4 ; %) and the eccentric (active: -41 ± 3 , inactive: -46 ± 3 ; %) muscular action as shown in Figures 1C and 1D. Differences between the percentage of maintenance of muscle strength in concentric peak torque are observed from set 5 between inactive groups (set 5: -19 ± 3 , set 6: -25 ± 3 ; set 7: -27 ± 3 , set 8: -31 ± 4 , set 9: -34 ± 4 , set 10: -41 ± 4 ; %) and active groups (set 5: -12 ± 2 , set 6: -15 ± 3 , set 7: -17 ± 3 , set 8: -19 ± 3 , set 9: -20 ± 3 , set 10: -23 ± 3 ; %).

Table 2 presents the hematological parameters. In spite of increases in all analyzed parameters, no significant difference was established between groups, before and following the fatigue protocol.

Table 1. Anthropometric parameters.

	Inactive	Active	Significance
Age (years)	26 ± 6	28 ± 7	$p = 0.636$
Height (cm)	1.74 ± 1.0	1.81 ± 1.0	$p = 0.057$
Weight (kg)	80 ± 13	87 ± 18	$p = 0.316$
BMI (kg/cm ²)	26 ± 3	26 ± 4	$p = 0.991$

Values expressed in mean ± standard deviation.

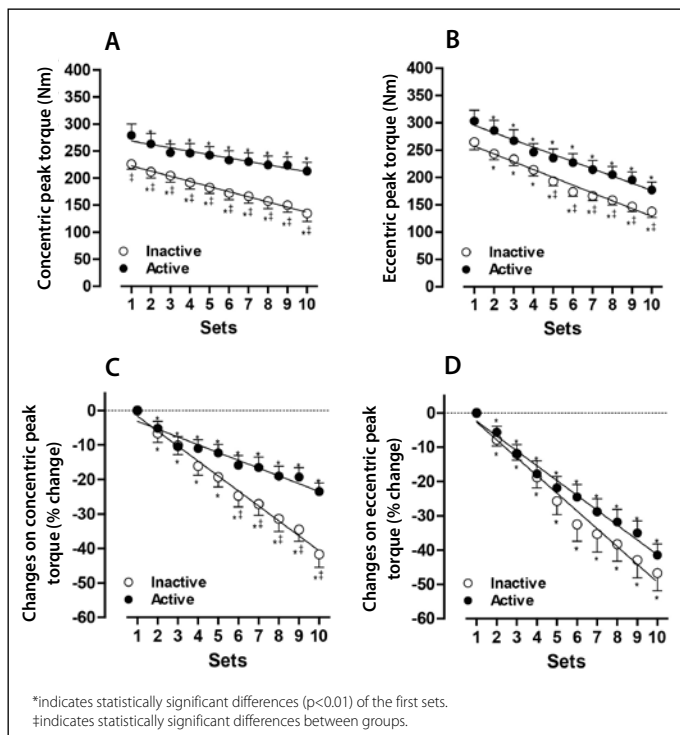


Figure 1. Values expressed as mean ± standard deviation of concentric peak torque (Panel A), Eccentric peak torque (Panel B), Changes (%) of concentric (Panel C) and eccentric (Panel D) peak torque during the muscle fatigue induction protocol of inactive (○) and active subjects (●).

Table 2. Hematological parameters.

	Inactive			Active		
	Before	After	%	Before	After	%
Hematocrit (%)	45.00 ± 0.01	47.00 ± 0.01	4 ± 1	45.00 ± 0.63	47.00 ± 0.01	5 ± 2
Red blood cells (count)	442 ± 40	487 ± 41	9 ± 4	477 ± 30	559 ± 37	10 ± 8
Leukocyte (cells/ μ L)	7565 ± 2878	8015 ± 4224	9 ± 58	6418 ± 3557	6632 ± 4460	1 ± 18

Values expressed as mean ± standard deviation.

DISCUSSION

The reduction in muscle performance has been the focus of numerous studies^{13,14} especially when considering the mechanisms involved in its development. When we refer to tension development, our data agree with the study previously published¹⁰ by our group, demonstrating that active individuals have higher fatigue resistance than inactive individuals. Taking into account the responses of different muscle actions, our data indicate a reduction of torque capacity in both groups in eccentric and concentric action. However, only significant differences were found between groups when comparing concentric actions.

The reduction in muscular strength (peripheral) is related to several mechanisms. These include: increase in concentrations of ADP, Pi, AMP, Ca²⁺ and H⁺, resulting in changes in sarcoplasmic reticulum function to release and recapture Ca²⁺, with decreased concentrations of phosphocreatine and glycogen. Also, reduced velocity of conduction and amplitude of action potentials due to the potassium flow, changes in threshold of excitability of muscle fibers for action potentials, and a delayed relaxation phase after a contractile movement are contributory factors^{13,15}. To our knowledge only a few studies^{7,9} investigated the differences between specific groups in response to fatigue.

Differences between muscle actions have already been investigated in the literature¹⁶⁻²¹ demonstrating greater impairment in concentric muscle activation^{3,19,20}, increased metabolic burden evaluated by lactate concentration³ in concentric muscle actions and of elastic components specifically in the titin protein^{16,21} have been recognized as indicators of this difference.

Considering hematological changes and physical exercise, many of the available studies have used horses²². To our knowledge, there are few studies²³ that investigated the effects of fatigue on hematological parameters in humans. In 1977 Novosadová²⁴ evaluated changes between rest and exercise and found no difference between the conditions. However, studies found significant changes in parameters such as blood viscosity⁷ and leukocyte profile²³.

The proportion of blood represented by red blood cells is called hematocrit. For adult men and women, the mean hematocrit concentration corresponds to 42 and 38 respectively, and these values can vary considering the presence of anemia, degree of bodily activity, and the altitude at which the person resides. In our study, the hematocrit-independent concentration of the groups has not changes significantly (Table 2) following the fatigue protocol. Additionally, the values found in our study were similar to others⁷. Thus, it is possible to consider the relationship between the variation in hematocrit concentration and the osmotic balance and cell hydration, which are characteristics present in predominantly anaerobic exercises⁷. Anaerobic performance demonstrates little change in water content and concomitant changes in blood viscosity when compared to aerobic exercises²⁵.

Such changes in viscosity with concomitant changes in red blood cells may contribute to alterations in the oxygen transport system but these alterations are found mainly in aerobic exercise²⁵. The primary function of red blood cells (erythrocytes) is the transportation of hemoglobin that carries oxygen from the lungs to the tissues. When free in the plasma of human beings, about 3% of total hemoglobin overflows through the capillary membrane into the interstitial space or through the kidney glomerular membrane to the glomerular filtrate. This happens each time that blood passes through the capillaries. Thus, hemoglobin should remain within the red blood cells to perform its functions effectively.

Red blood cells have other functions besides hemoglobin transport. For example, they contain a large amount of carbonic anhydrase, an enzyme that catalyzes the reversible reaction between carbon dioxide and water to form carbonic acid, increasing by thousands of times the

velocity of this reaction²⁶. The velocity of this reaction helps transport large quantities of CO₂ in the form of bicarbonate ions from the tissues to lungs, where it is converted to CO₂ and eliminated to the atmosphere as a product of body metabolism²⁶. The hemoglobin in the cells is considered an excellent basic acid buffer, and responsible for most of the blood acid-base buffering capacity.

The figures for red blood cells in the present study did not differ between active and inactive individuals (Table 2), and were similar to other studies in humans²⁷ and animals²⁸. However, values remain indifferent when referring to anaerobic exercises, especially when performed close to exhaustion.

When referring to leukocyte concentration, no differences were noted after completion of the fatigue protocol in both groups (Table 2). Leukocytes are formed partly in the bone marrow (granulocytes, monocytes and some lymphocytes), and partly in the lymphatic tissue (lymphocytes and plasma cells). The leukocyte action is directed at areas of severe infection and inflammation, promoting fast and powerful defense mechanisms against infectious agents.

The increased concentration of leukocytes is closely related to increased inflammation and serious infections²⁹. When referring to physical exercise, especially depending on intensity and duration several Dias et al.³⁰ showed increase, decrease or maintenance of leukocyte values. Although not statistically different, the leukocyte values of inactive individuals were higher than those of active individuals, possibly as a result of the immune protection promoted by regular practice of physical

activity³⁰. Studies^{23,27,29} have showed that in acute situations, the practice of strenuous exercise can increase the inflammatory process allowing increased leukocyte content. Considering data from our study, we did not demonstrate significant increases in leukocyte content, which may indicate absence of inflammation following the fatigue protocol. However, the values in this study were similar to other³⁰. Additionally, the increase of lymphocytes is controlled by catecholamine concentration, particularly in activities of short duration³¹.

CONCLUSION

In conclusion, the neuromuscular response differs when analyzing eccentric and concentric actions. However, the concentric action had more impact on the development of muscle strength during the fatigue protocol, which was different between active and inactive individuals. Additionally, no hematological changes were found following the fatigue protocol in both groups.

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