MUSCLE DAMAGE AND IMMUNOLOGICAL PROFILE IN THE IRONMAN BRAZIL TRIATHLON

EXERCISE AND SPORTS MEDICINE CLINIC

ORIGINAL ARTICLE

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ABSTRACT

Introduction: The Ironman triathlon is characterized as an activity of long duration in which acute organic changes are present. The objective of this study was to investigate the relationship between muscle damage and immune system. Methods: Blood samples were obtained from 21 athletes at three different times: 2 days before (pre), immediately after (post) and 6 days after the event (6 days post). The creatine kinase (CK), total leukocytes, lymphocytes, lymphocyte subtypes CD4+ and CD8+ variables were independently assessed, as well as the CD4+ / CD8+ ratio. Additionally, the CK correlation as a marker of muscle damage with the other variables was studied. Results: Significant results observed were: leukocytes pre (6,242.9 mm3; SD: 1,233.3) and post (mean: 18,398.1 mm3; SD: 3,904.0; p< 0.0001), post (mean: 18,398.1 mm3; SD: 3,904.0) and 6 days post event (mean: 6,396.4 mm3; SD: 1299.8; p< 0.0001), CK pre (mean: 173.2 U/l; SD: 103.7) and post (2339.4 U/l; SD: 1729.0; p< 0.0001), CK pre (mean: 173.2 U/l; SD: 103.7) and 6 days post (mean: 368.1 U/l; SD: 274.4; p< 0.0053), CK post (mean: 2339.4 U/l; SD: 1729.0) and 6 days post (mean: 368.1 U/l; DP: 274.4; p< 0.0003), CD4+ / CD8+ pre (mean: 1.9; SD: 0.8) and 6 days post (mean: 2.4; SD: 1.1: p<0.00032). Conclusion: There was muscle damage immediately after the event and improvement in immune profile on the sixth day after the event.

Keywords: creatine kinase, lymphocytes, leukocytes, triathlon.

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INTRODUCTION

The *Ironman* triathlon is a long duration event of 226 km, composed of 3.8 km of swimming, 180 km of cycling and 42.195 km of running with duration range between a bit more than eight hours, which is the time of the first places, and 17 hours, maximal permitted time to finish the event.

The Brazilian phase of the Ironman has been held in Florianópolis, the capital city of Santa Catarina state, since 2001, when 498 athletes participated in the event. Since then, the event has exponentially grown and in 2011 the total number of participants was 1,822. However, the number of enrollments does not supply the real demand, since the 2,000 enrollments made available for 2012 finished in only 14 minutes, which left many athletes out.

In events with duration longer than four hours, organic and psychological alterations are factors which present great influence on the athletic performance and in the onset of health problems[1,2]. Previous studies have demonstrated the influence of long- duration physical exercise in the immune function, including populations of leukocytes, T lymphocytes and natural killer cells (NK)[3,4], and induction of muscle damage and systemic inflammatory response[5,6].

Muscle damage, verified in prolonged activities, such as the *Ironman* triathlon, is aassociated with increase of the CK levels[7], interleukins release (IL)s, and consequently modulation of the immune system. It is suggested that immunological and hormone alterations occur due to training of great volume and intensity, which, associated with the stress caused by the competition, may be perceived after the event[8]. These alterations may cause immunodepression[9], with change in the number of neutrophils and alterations in the proportion of the subtypes of lymphocytes, being able to cause increase of the susceptibility to infections, commonly in the upper respiratory tract[9]. The aim of this study was to verify the onset of muscle damage and its relation with muscle damage and its correlation with the immunological profile in the *Ironman* triathlon Brasil athletes.

METHODS

Initially, 23 amateur athletes members of a triathlon team from Florianópolis, with two female and 21 male subjects, were invited to participate in the study. During the collection period, to individuals were excluded due to have missed two out of the three scheduled blood collections.

The present research project was approved by the Ethics Committee of the institution where the study was conducted. A post-clarified consent form was presented to the subjects according to protocol from 16/05.

Blood collections – Blood collection of 10 ml was performed in three distinct moments: two days before the event (pre), immediately after the end of the event (post) and six days after the event (six days after). 21 athletes out of the total of participants were submitted to blood collection only in the first and second moments and 14 athletes in the three moments.

Venous puncture, storage, transportation and method – the venous puncture was performed through vacuum collection in the cubital fossa using *Sarstedt* collection tubes with EDTA K_2 anticoagulant – volume 1.2 ml; *Sarstedt* collection tubes without SST anticoagulant serum gel – volume 4.9 ml; *Sarstedt* needles of multiple collection. After collection of the biological material and the

necessary time for blood coagulation (minimum of 30 minutes), the serum gel tubes were centrifuged during 15 minutes at 1.500 rotations per minute in a calibrated centrifuge. Subsequently they were stores in a refrigerator and carried in a suitable thermal bag with recycled ice to be evaluated on the same day. The hemogram was analyzed for leukocytes evaluation through: sample – total blood (with EDTA anticoagulant); – SYSMEX-2100D device, which is an automatic hematologic analyzer. The fluorescent flow cytometry system is applied in that device, which presents excellent differential system of the white series.

Determination of total creatine kinase (CK total) – Serum sample, by enzymatic method, through the Advia System – 1650 Bayer. The reference values for the total CK at temperature of 37°C for women is of 25 at 165 U/l, and for men, it is between 26 and 190 U/l.

Determination of the $CD4^+$ and $CD8^+$ lymphocytes levels – Sample: total blood (with EDTA anticoagulant). Through the use of monoclonal antibodies and through flox cytometry, the presence of cellular and intracellular membrane antigens which are important in the study of the cells and characterize the different lymphocyte populations in relative terms, their function, with the aim to aid in diagnosis and follow-up of immunodeficiency.

Statistical analysis – It consisted of a descriptive part, with determination of descriptive statistics and an inferential part, which tested occasional significant difference between the performed exams. Descriptive statistics: determined the arithmetic mean and standard deviation. Inferential analysis: verified occasional correlations between some analysis parameters, through Pearson correlation. The significance level of the test was of 95% ($p < 0.05$) and the statistical analyses were developed with the use of the Statistica program, version 7.0.

RESULTS

The studied sample was composed of 21 athletes, among which, two were women and 19 men. Mean age was of 37.1 ± 7.9 years, and minimum age of 21 years and maximum of 51 years. Mean time of the event for the participants in the study was of 12h00' ± 1h30', with the shortest time being 9h36' and the longest 6h36'.

The behavior of the creatine kinase, leukocytes, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ and lymphocytes variables before, immediately after and six days after competition is presented in table 1. The statistical tests (*Student's t test*) measured the statistically significant differences between the estimations of the means of the parameters compared in the three situations two by two.

The mean variations of the studied parameters in the different moments are illustrated in figure 1.

There was statistically significant difference in the CK evaluation in the three situations, pre with post ($p < 0.0001$), pre with six days post ($p = 0.0053$) and post with six days post ($p = 0.0003$). The leukocytes presented statistically significant difference in two situations, pre with post ($p < 0.0001$) and post with six days post ($p < 0.0001$). The $CD4^+/CD8^+$ ratio presented statistically significant difference between pre and six days post ($p = 0.0032$).

The results of the correlation analysis between the percentage of CK variation with total leukocytes, $CD4^+/CD8^+$ and lymphocytes are presented in table 2.

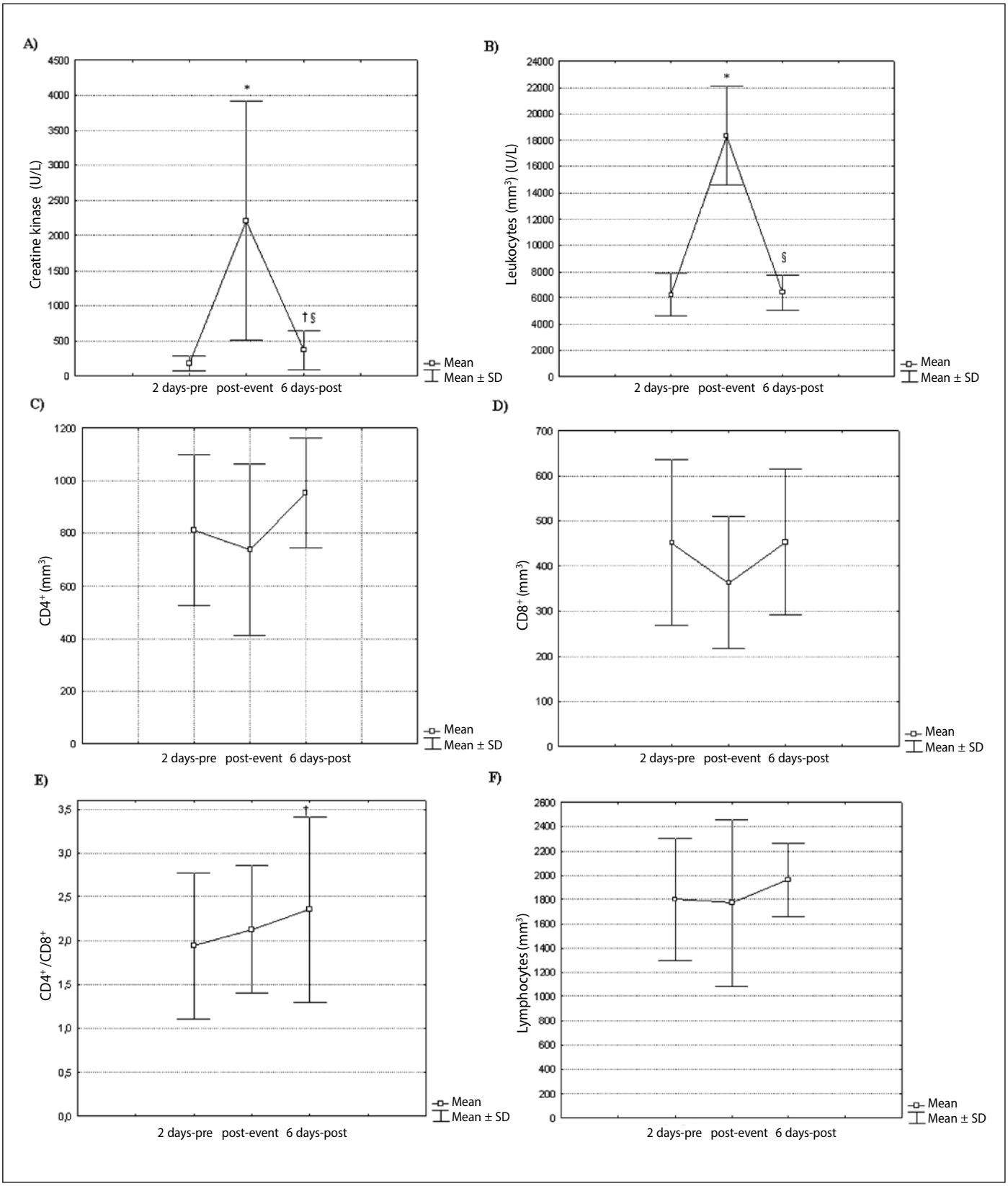
Table 1. Plasma values of creatine kinase, leukocytes, CD4+, CD8+, CD4+/CD8+ and lymphocytes.

Variable	n	Pre		Post		6 days post		p value
Creatine kinase (U/l)	21	173.24	(103.72)	2.339.43	(1.728.99)			<0.0001
	14	179.71	(124.79)			368.07	(274.44)	0.0053
	14			2.103.93	(1.594.65)	368.07	(274.44)	0.0003
Leukocytes (mm^3)	21	6.242.86	(1.666.02)	18.398.10	(3.904.05)			<0.0001
	14	6.464.29	(1.695.91)			6.396.43	(1.299.80)	0.8663
	14			18.185.00	(4.266.31)	6.396.43	(1.299.80)	<0.0001
$CD4^+$ (mm^3)	21	810.48	(287.27)	766.10	(323.75)			0.5261
	14	877.43	(275.17)			952.79	(209.17)	0.2451
	14			803.79	(292.78)	952.79	(209.17)	0.0729
$CD8^+$ (mm^3)	21	452.52	(183.44)	378.38	(143.64)			0.0710
	14	483.86	(209.40)			454.07	(161.37)	0.3675
	14			394.36	(154.74)	454.07	(161.37)	0.2207
$CD4^+/CD8^+$	21	1.94	(0.84)	2.11	(0.73)			0.1274
	14	2.02	(0.90)			2.36	(1.06)	0.0032
	14			2.19	(0.78)	2.36	(1.06)	0.3124
Lymphocytes (mm^3)	21	1.801.37	(503.78)	1.833.06	(684.79)			0.8105
	14	1.927.30	(417.09)			1.960.32	(299.35)	0.7101
	14			1.938.23	(619.85)	1.960.32	(299.35)	0.9027

Values are means (standard deviation). Pre = two days before the event; Post = immediately after the event ; 6 days Post= six days after the event.

DISCUSSION

Muscle pain, as well as gait limitation, are referred symptoms which make the athlete look for help in the medical kiosk after the event. Such situation derives from muscle damage which may be associated with many factors[10]. Studies have assessed muscle damage markers as pain and plasma muscular enzymes. It is believed that these parameters do not accurately measure the extension of the muscle damage[11], and that flexibility and power parameters are more efficient methods to evaluate muscle damage[11], being the absence of measurement of these variables a limitation of this study. However, the CK, lactate dehydrogenase (LDH), troponin-I and myoglobin are frequently described as muscle damage markers, since these cytoplasmic molecules do not have the ability to surpass the sarcoplasmic membrane barrier[12]. In the performed study, the measurement of the CK enzyme was used since it is considered the best indirect biomarker of damage to the muscle tissue[12]. The muscle damage, on its turn, stimulates the inflammatory process through the activation of interleukins (IL's), which modulate the activity and distribution of the immunological cells[12]. In a study conducted with ultramarathoners, significant alterations were verified in biomarkers such as the CK and inflammation markers as IL-6 and C-reactive protein, suggesting hence, that the inflammatory process is associated with muscle damage[13]; additionally, there is evidence of strong positive correlation between the increase of



2 days Pre = two days before the event; Post-event = immediately after the event; 6 days Post = six days after the event. A) Creatine kinase variation (CK); B) Leukocytes variation; C) CD4+ activity variation; D) CD8+ activity variation; E) CD4+/CD8+ ratio; F) Lymphocytes variation. * significant variations between pre and post-event; † significant differences between pre and six days after the event; § significant differences between post-event and six days after the event; $p < 0.05$.

Figure 1. Mean variations of the plasma values of creatine kinase , leukocytes, CD4+, CD8+, CD4+/CD8+ and lymphocytes.

IL-6 and plasma CK[14]. Moreover, it is acknowledged that the muscle contraction and damage stimulate the synthesis of IL-6[15] and its effects modulate the immunological and metabolic aspects.

Consequently, it is believed that the modulation of the profile of the immunological cells suffer influence of the inflammatory process caused by the muscle damage. However, other factors, associated with or independent from muscle damage, such as nutritional status and use of antioxidants may influence on the interleukins responses[16-19]. In the present study, these factors were not controlled, which becomes a limitation of it.

Table 2. Correlation of the percentage variations of the creatine kinase with total leukocytes, CD4+/CD8+ and lymphocytes.

			Leukocytes		$CD4^+/CD8^+$		Lymphocytes	
			Δ% pre post	Δ% pre 6 post	Δ% pre post	Δ% pre 6 post	Δ% pre post	Δ% pre 6 post
Creatine kinase	Δ% pre / post	R	0.33	–0.34	0.05	–0.27	0.09	0.11
		p value	0.1490	0.2380	0.8280	0.3500	0.6960	0.7020
	Δ% pre / 6 post	R	–0.12	–0.48	–0.20	–0.23	0.20	0.24
		p value	0.6780	0.0800	0.4830	0.4370	0.4950	0.4180

* $p < 0.05$.

When the muscle damage is evaluated through the CK enzyme, significant increase of the mean serum levels of this enzyme immediately after the event was observed (second moment) and remained high, with statistical significance; on the sixth day after the event (third moment) in relation to the two days previous to the event (first moment) – figure 1A. Such enzymatic behavior reflects the persistence of the tissue injury and, consequently, of inflammatory activity. The findings agree with Neubauer *et al.* (2008), who investigated 42 *Ironman* triathletes, in the following moments: two days before, immediately after, one, five and 19 days after the event. This study verified significant relations among the leukocyte dynamics, muscle damage and interleukins. Remarkable initial inflammatory response was evidenced, which generally rapidly decreases; however, systemic inflammation of low degree remains at least until the fifth day after the event, possibly reflecting incomplete muscular recovery[6].

In acute physical exercise, the inflammatory response consequent of the muscle damage, hormone alterations and cardiac debt promote the demargination of leukocytes from the endothelial bed and release of bone marrow cells, which results in the exponential increase of the serum concentration of leukocytes[20]. Leukocytosis was observed in the present study immediately after the event and six days post (figure 1B), presenting behavior similar to the CK. Such fact corroborates another study[21] in which direct correlation of leukocytosis as marker of muscle tissue damage was observed. The total leukocytes levels in the third moment were similar to the baseline levels, being probable that on the sixth day post event they do not suffer influence of hemodynamic and neuroendocrine alterations in a remarkable way. The correlation between CK percentage variation and leukocytes in these moments was not statistically significant (table 2), which may be explained by different moments of plasmatic peaks of these variables or by a small study sample, being hence, a limitation of the study.

The peripheral blood lymphocytes consist of subtypes of T-cells, B-cells and NK cells. The alterations in the number of total circulating lymphocytes and T subtypes $CD4^+$ and $CD8^+$ are presented as a two-phase characteristic in which the increase of the circulating lymphocytes (lymphocytosis) occurs during or immediately after the physical exercise, followed by decrease (lymphocytopenia) during the initial recovery stage and return to baseline resting levels. Such alterations are proportional to the exercise intensity and duration[8]. The results in this study did not verify this behavior, probably due to the studied moments. On the other hand, the total lymphocytes did not present statistically significant alterations among the three investigated moments (figure 1F), being these findings in agreement with another study[22]. Moreover, statistically significant correlation has not been observed between the CK percentage variation and total lymphocytes in the different moments, denoting that the muscle damage did not influence on the circulating levels of lymphocytes.

The $CD4^+$ T lymphocytes did not presente statistically significant difference in the different moments, remaining unchanged after the long-duration exercise (figure 1C), which agreed with the findings of other authors[23,24].

The unproportional alteration between subtypes of T $CD4^+$ and T $CD8^+$ cells results in alteration in the $CD4^+/CD8^+$ ratio, which commonly declines during and after exercise. This ratio has been used as a useful index which represents the relative distribution of the T lymphocyte subtypes[25], suggesting that decrease in the $CD4^+/CD8^+$ ratio may be associated with the suppression of the responsiveness of the T cells after exercise[26], consequent immunodepression and susceptibility to infections. Some authors found that moderate training of long duration does not change the $CD4^+/CD8^+_{\ ratio}$ [27,28]. On the other hand, endurance exercise (75% VO_{2max}) decreases the concentration of $CD4^+$ cells, or even lead to increase in the $CD8^+$ lymphocytes concentration [29], resulting in decrease in the $CD4^+/CD8^+$ ratio. However, although some studies have demonstrated decrease in the $CD4^+/CD8^+$ balance, infection of upper airways (UAII) episode was not observed in these athletes[30], suggesting that the body is at potential status of alertness against external aggression, which tends to take the clinical meaning of the transitory decrease of the $CD4^+/CD8^+$ ratio for granted. In the present study, it was verified that there was no statistically significant difference in the CD4+/CD8+ T lymphocytes ratio between two days before and immediately after the event; however, statistically significant increase of the CD4+/CD8+ ratio was observed between two days before and six days after the event (figure 1E). Such fact did not denote tendency to immunossupression in the immediate post-event, and suggests increase of the immunological power six days post, probably due to the exercise characteristic of moderate intensity, despite the long duration.

In addition to that, no statistically significant correlation between the CK variation percentage and $CD4^+/CD8^+$ T lymphocytes ratio and total lymphocytes was observed among the different moments (table 2), which does not let us associate in this study muscle damage injury with alterations in the distribution and absolute number of circulating lymphocytes.

To sum it up, exercise of long duration and moderate intensity caused evidence of muscle damage and of alterations in the immunological profile, observed by the behavior of the CK and leukocytes levels, besides the $CD4^+/CD8^+$ ratio.

CONCLUSION

The study evidence serum enzymatic alterations which let us conclude the onset of muscle damage after the Ironman Brasil triathlon event.

The muscle damage observed did not correlate with the alterations in the profile of the studied immune cells.

An alert or boosted immunological state may be established six days after the *Ironman* triathlon event.

In Ironman athletes, complementary studies evaluating muscle damage, inflammatory mediators and modulation of immune cells in different moments are necessary.

All authors have declared there is not any potential conflict of interests concerning this article.

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