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 < 0.001), post (mean: 18,398.1 mm3; SD: 3,904.0) and 6 days post event (mean: 6,396.4 mm3; SD: 1,299.8; p < 0.0001), CK pre (mean: 173.2 U/l; SD: 103.7) and post (2339.4 U/l; SD: 1,729.0; p < 0.0001), CK post (mean: 2339.4 U/l; SD: 1,729.0) and 6 days post event (mean: 6,396.4 U/l; SD: 1,299.8; p < 0.0001), CK post (mean: 2339.4 U/l; SD: 1,729.0) and 6 days post event (mean: 6,396.4 U/l; SD: 1,299.8; p < 0.0001), CK pre (mean: 173.2 U/l; SD: 103.7) and post (mean: 368.1 U/l; SD: 274.4; p < 0.0053), CK post (mean: 368.1 U/l; SD: 274.4; p < 0.0053), CD4+ / CD8+ ratio (mean: 1.9; SD: 0.8) and 6 days post (mean: 1.9; SD: 0.8) and 6 days post (mean: 1.9; SD: 0.8). 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.

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. 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), and induction of muscle damage and systemic inflammatory response.

Muscle damage, verified in prolonged activities, such as the Ironman triathlon, is associated with increase of the CK levels, interleukins release (ILs), 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. These alterations may cause immunodepression, 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.

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 K3 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
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' ± 2h37'.

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 flow 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 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.

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. 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, and that flexibility and power parameters are more efficient methods to evaluate muscle damage, 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. 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. The muscle damage, on its turn, stimulates the inflammatory process through the activation of interleukins (ILs), which modulate the activity and distribution of the immunological cells. 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, additionally, there is evidence of strong positive correlation between the increase of

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Pre</th>
<th>Post</th>
<th>6 days post</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine kinase (U/l)</td>
<td>21</td>
<td>173.24 (103.72)</td>
<td>2.339.43 (1.728.99)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>179.71 (124.79)</td>
<td>368.07 (274.44)</td>
<td>0.0053</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.103.93 (1.594.65)</td>
<td>368.07 (274.44)</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Leukocytes (mm³)</td>
<td>14</td>
<td>2.422.86 (1.666.02)</td>
<td>18.398.10 (3.904.05)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>4.646.29 (1.695.91)</td>
<td>6.396.43 (1.299.80)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

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Values are means (standard deviation). Pre = two days before the event; Post = immediately after the event; 6 days Post = six days after the event.
IL-6 and plasma CK. Moreover, it is acknowledged that the muscle contraction and damage stimulate the synthesis of IL-6 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. In the present study, these factors were not controlled, which becomes a limitation of it.
Such fact corroborates another study \(^{21}\) in which direct correlation was observed in the present study immediately after the event increase of the serum concentration of leukocytes \(^{20}\). Leukocytosis and release of bone marrow cells, which results in the exponential promote the demargination of leukocytes from the endothelial bed

**Quentin**

**of the muscle damage, hormone alterations and cardiac debt**

**of leukocyte dynamics, muscle damage and interleukins.** Remarkable

**moments: two days before, immediately after, one, five and 19 days**

**Ironman triathletes, in the following (2008), who investigated 42**

**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 present 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\% \(V_{O2\max}\)) 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 immunoosuppression 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.

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

<table>
<thead>
<tr>
<th>Creatine kinase</th>
<th>Leukocytes</th>
<th>CD4+/CD8+</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\Delta%) pre / post R</td>
<td>(\Delta%) pre / post P</td>
<td>(\Delta%) pre / post R</td>
</tr>
<tr>
<td>(%) pre / post</td>
<td>0.33</td>
<td>-0.34</td>
<td>0.05</td>
</tr>
<tr>
<td>p value</td>
<td>0.1490</td>
<td>0.2380</td>
<td>0.8280</td>
</tr>
<tr>
<td>(%) pre / 6 post</td>
<td>-0.12</td>
<td>-0.48</td>
<td>-0.20</td>
</tr>
<tr>
<td>p value</td>
<td>0.6780</td>
<td>0.0800</td>
<td>0.4830</td>
</tr>
</tbody>
</table>

* \(p < 0.05\)
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.

REFERENCES