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original article

Impact of ironman triathlon on oxidative stress parameters

Impacto do triatlon ironman sobre os parametros de estresse oxidativo

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Abstract – Several studies have investigated the biochemical response changes that take place in Ironman triathletes, but there are few data on oxidative stress changes. The objective of this study was to investigate oxidative stress parameters in triathletes after an Ironman event. The sample consisted of eighteen male triathletes, with a mean age of 34.5 ± 2.15 years, weight 69.3 ± 1.9 kg, and height 1.71 ± 0.18 m. The Ironman triathlon consists of a 3.8-km swim, a 180-km bicycle ride, and a 42.2-km (marathon) run. Before the competition and immediately after its conclusion, 10-mL blood samples were collected, centrifuged and frozen at -80ºC for subsequent analysis. Total antioxidant capacity, lipid peroxidation, protein carbonylation, and total thiol content were measured. The results showed a significant increase in all markers after the event ($p<0.05$) in relation to the pre--event period, which conclusively shows that the Ironman triathlon induces significant changes oxidative stress markers in athletes and that antioxidant supplementation would be important to reverse these effects.

Key words: Oxidative stress; Physical exercise; Reactive oxygen species.

Resumo – *Vários estudos têm investigado as alterações na resposta bioquímica em triatletas participantes de provas de Ironman, mas poucos dados relatam as mudanças de estresse oxidativo. O estudo teve como objetivo investigar os parâmetros de estresse oxidativo em triatletas após corrida de Ironman. Participaram do estudo, dezoito triatletas do sexo masculino, com idade média de 34,5 ± 2,15 anos, peso 69,3 ± 1,9 kg e altura 1,71 ± 0,18 m participaram do estudo. A corrida de Ironman consiste em 3,8 km de natação, 180 km de bicicleta e 42,2 km de corrida. Antes da corrida e imediatamente após seu término foi retirado 10 mL de sangue, sendo o mesmo centrifugado e armazenado o soro em freezer -80ºC para posteriores análises. A capacidade antioxidante total, lipoperoxidação, carbonilação de proteínas e conteúdo total de tióis foram determinadas. Os resultados mostraram um aumento significativo na quantidade de hidroperóxidos, carbonilação de proteínas e uma redução na capacidade antioxidante total do plasma e no conteúdo total de tióis após a prova ($p<0.05$) em relação à pré-prova, concluindo que a prova de Ironman provoca alterações significativas nos marcadores de estresse oxidativo em atletas e que uma suplementação com antioxidantes seria importante para reverter estes efeitos.*

Palavras-chave: *Espécies reativas de oxigênio; Estresse oxidativo; Exercício físico.*

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INTRODUCTION

Ironman triathlon consists of a sports competition including three different sports in one single event (3.8 km swim, 180 km bike ride, and 42.2 km run), raced in this order and without a break, during which athletes exercise for a long period of time. These competitions require high resistance and can lead to heat stress and dehydration[1,2], muscle injury[3], oxidative stress, and inflammation[4,5].

Among the biochemical and physiological changes affecting ironman athletes, researchers have focused their attention on oxidative stress[6,7]. The consumption of oxygen for aerobic production of adenosine triphosphate (ATP) may increase from 10 to 20 times during exercises compared with resting levels[8] and up to 100 times when referring to muscles[9]. This causes a concomitant increase in the production of reactive oxygen species (ROS) and subsequent oxidative damage to cellular structure because of oxidation of membrane lipids, protein carbonylation, carbohydrate oxidation, and damage to nucleic acids[9]. In addition, other phenomena may prompt the production of ROS during long sports competitions, such as migration of polymorphonuclear cells to the injured tissue and ischemic muscle response[5].

According Cruzat et al.[10], individuals who undergo intense and prolonged exercise or exhaustive training, or even those with very high training frequency may exceed the capacity of endogenous antioxidant system and, consequently, cause severe muscle injuries, with subsequent inflammation and oxidative stress. Therefore, high rates of oxidative stress may contribute to decreased performance, fatigue, muscle damage, and muscle pain[11,12]. Although studies have described the process of production of ROS during exercise[13,14] and the biochemical changes in prolonged exercises, there is need to broaden the current knowledge on the impact of this type of exercise on oxidative stress parameters, especially in terms of Ironman races, mainly because in this type of sports there are many different variables that can affect athletes' performance, such as weather, athletes' current health status, diet, level of training, and duration of race.

In endurance races, the athletes' physical wear causes a significant energy demand and substantial change in the antioxidant defense mechanisms and reduced cellular repair system[5]. As a result, some studies suggest an increase in oxidative damage of biomolecules due to increased ROS generated mainly via the mitochondrial respiratory chain, incomplete reduction of oxygen, as well as decrease in antioxidant defense system[13,15].

The literature provides only assumptions that need further investigation. Thus, the objective of the present study was to investigate the impact of ironman triathlon on oxidative stress parameters.

METHODS

Sample

Thirty-one males who competed in the 2008 Ironman Florianopolis Triathlon, Brazil, were invited to participate in this study. Inclusion criteria were

as follows: participants could not be using any medications, antioxidants or dietary supplements, should be non-smokers, and could not have any symptoms of illness in the previous 48 hours, such as colds and fever. Of the individuals selected, only nineteen triathletes who completed the race in less than 11 hours were included in the study. Athletes' characteristics are shown in Table 1. Participants signed a consent form. The study was approved by the local Research Ethics Committee. Athletes were allowed to eat and drink freely during the race.

Table 1. Athletes' body mass (kg) and creatine kinase (U/L) before and after the Ironman race

	Before the race	After the race
Age (years)	32.4 (3.09)	
Height (m)	1.71 (0.18)	
Body mass (kg)	67.9 (3.4)	66.4 (1.8)
Creatine kinase (U/L)	161.8 (21.32)	2231.5 (239.7)*

Values are shown as mean±standard error of mean. The significant difference between the means (*) was $p < 0.05$.

Race

The race consists of 3.8 km swim, 180 km bike, and 42.2 km run. Environmental conditions varied from 20 to 25 ºC and 79-85% relative humidity.

Data collection

Upon arrival at the laboratory before the race, participants had their height and weight measured, and a 10-mL blood sample was collected from an antecubital vein. Up to 20 minutes after the end of the race, another 10-mL blood sample was collected. Blood was collected using heparinized plastic tubes and centrifuged at 1500 rpm for 10 min at 4 °C. Aliquots of erythrocyte and plasma samples were stored at -80 °C for later biochemical analysis. A mobile laboratory was used during the sports event to ensure proper collection, identification, and storage of samples.

Biochemical Tests

- Total Antioxidant Capacity

Athletes' plasma antioxidant capacity was assessed using the TRAP technique (Total Radical Trapping Antioxidant Potential, Lissi et al.[16]). Twenty µl of plasma were added to 4 ml of peroxyl radical generating system (10 mM AAPH, 4 mM luminol, 100 mM glycine buffer, 8.6 pH) and plasma chemiluminescence was monitored and recorded for 50 minutes using the Wallac 1409 DSA Liquid Scintillation Counter (Wallac Oy, Turku, Finland). Two parameters were evaluated in this test: total antioxidant reactivity (TAR), calculated according to Lissi et al.[17], and reaction over time, represented as the area under the curve (AUC) calculated using a software (GraphPad Software Inc., San Diego, CA, USA - version 5.00), according to Dresch et al.[18].

- Oxidative Damage

Formation of hydroperoxide plasma was established as a general marker of oxidative damage in lipids. The amount of hydroperoxide was determined by spectrophotometry at 550 nm and data were expressed as nmol/mg protein as previously described by Hermes-Lima et al.[19]. Damage to proteins was determined by the formation of carbonyl groups based on the reaction with dinitrophenylhydrazine. Carbonyl content was determined by spectrophotometry at 370 nm using a coefficient of $22.000M^{-1}$. Data were expressed as nmol/mg protein as previously described by Levine et al.[20]. Total thiol content was determined in a reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB. The content was read spectrophotometrically at 412 nm and expressed in nmol of DTNB/mg protein[21].

- Protein Determination

The amount of protein in the lipid and protein oxidative damage tests was determined according to the technique of Lowry et al.[22].

Statistical treatment

All data are presented as mean and standard error of mean (SEM) and t-test was used to test differences between pre-race and post-race. P-value < 0.05 was used to determine statistical significance using the Bonferroni post-hoc test. Normality was evaluated by means of the Smirnoff-Kolmogorof test.

RESULTS

- Total Antioxidant Capacity

According to figures 1A and 1B, athletes had a lower plasma antioxidant capacity after the race when compared with the values shown before the race. TAR value before the race was higher than the post-race value, 27.37±7.31 and 10.93±1.83 nM trolox indicating that the samples collected before the race have a higher antioxidant capacity than the samples collected after the race. Before the race, the mean AUC was 194.02±22.94 mM trolox, while the post-race AUC was 279.56±17.32 mM trolox. AUC value is inversely proportional to the antioxidant capacity of the sample, so that the group with lower AUC is the one with higher antioxidant potential.

- Oxidative damages

According to Figure 2A, 2B and 2C, the results show an increase in the amount of hydroperoxides formed after the Ironman race (pre-race = 0.74±0.2 nmol/mg protein; post-race = 1.4±0.2 nmol/mg protein); protein carbonylation (pre-race = 2.2±0.4 nmol/mg protein; post-race = 5.8±1.8 nmol/mg protein) and a reduction in the total thiol content (pre-race = 27.8±4.2 nmol DTNB/mg protein; post-race = 18.1±6.4 nmol DTNB/mg protein).

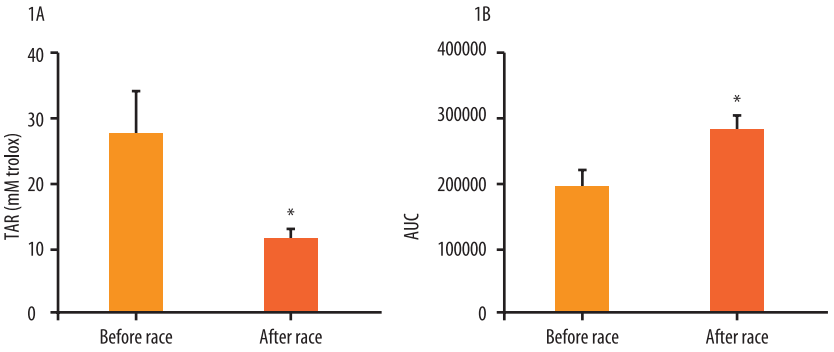

Figure 1. Athletes' total plasma antioxidant potential before and after the Ironman race shown as TAR (A) and AUC (B). Data expressed as mean±standard error of mean; *significant statistical difference for t test; p = 0.0253 and p = 0.0178, respectively.

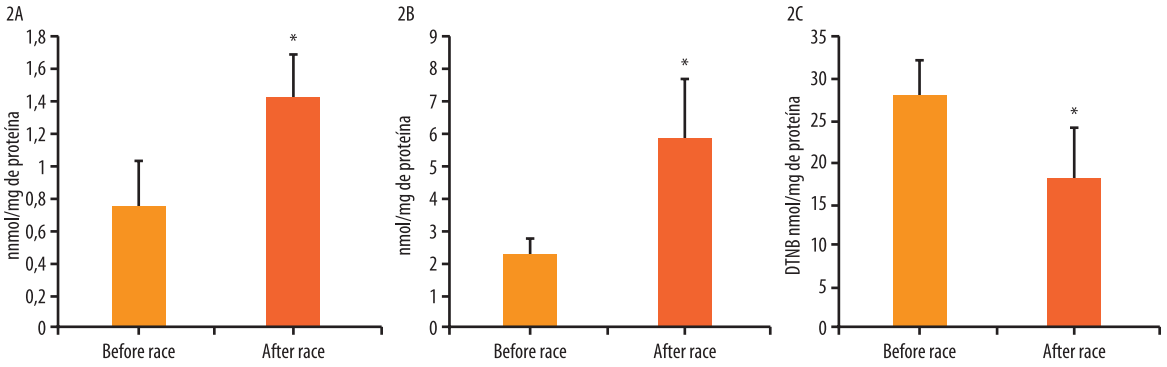

Figure 2. Athletes' plasma oxidative damage before and after the Ironman race, represented by lipid peroxidation (A), protein carbonylation (B), and total thiols (C). Data expressed as mean±standard error of mean; *significant statistical difference for t test; $p < 0.05$.

DISCUSSION

The production of ROS represents a normal physiological process, however, during strenuous exercise, there is an increase in the production of these species with a reduction of the defense system, leading to oxidative stress. Excessive production of these species and consequent oxidative damages have been associated with decreased performance, fatigue, muscle damage, and "overtraining"[23,24]. Although strenuous exercise promotes increased production of ROS and oxidative damage[6,4], studies have shown that endurance training increases the antioxidant defense system, as well as muscle oxidative capacity[24,25]. However, these positive effects of training may not be sufficient to reduce oxidative damage caused by intense exercise of long duration.

The increase we found in creatine kinase activity after the Ironman race (Table 1) suggests the presence of muscle microlesions and a possible ultrastructural disruption of the sarcolemma[26]. Similar results were observed in athletes who participated in one of the main ultraendurance cycling races, the Tour de France[27].

Metabolic and muscle wasting in ironman athletes promotes a significant increase in the production of ROS. Probable mechanisms for the increased production of ROS during and after ultraendurance exercises,

such as the Ironman race, include deviation of electron flow through the mitochondrial respiratory chain during oxygen metabolism, increased activity of xanthine oxidase triggered by hypoxia, increased activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in inflammatory processes, increased activity of phospholipase, as well as auto-oxidation of heme proteins[15].

Due to the possible increase in the production of ROS, our results show a decrease in the TRAP in plasma levels as seen in Figure 1. The TRAP is a method that quantifies the plasma antioxidant potential and its decrease after the race suggests an increased production of ROS, which can lead to significant oxidative damage. The low levels of the TRAP found after the Ironman race and the increased AUC shown in Figure 1A and 1B may be mainly associated with the oxidation of glutathione induced by exercises. Pinho and colleagues[25] suggest that increased oxidation is caused by excessive production of ROS after intense exercise. Glutathione is one of the most important antioxidants in biological systems because it is a direct scavenger of free radicals, glutathione peroxidase substrate and it can also be involved in the reduction of other antioxidants such as vitamin E and C[11]. Thus, depletion of glutathione reduces the capacity of antioxidant defense leading to oxidative stress.

The good level of fitness based on the duration of the race (less than 12 hours), seems to be insufficient to prevent oxidative damage to lipids (Figure 2A) and proteins (Figure 2B and 2C), although high intensity training is able to increase plasma antioxidant defenses[25]. Studies conducted by Child and colleagues[28] show different results. Athletes participated in a simulated half-marathon race and showed an increase in serum total antioxidant capacity; however, exercise induced increased concentrations of malondialdehyde. Mastaloudis et al.[29] found an increase in total antioxidant capacity after endurance races and they suggest that this response should be related to a higher release of antioxidants. It is possible that such differences among the studies may be related to the characteristics of the exercise, the athletes' fitness, or even the methodology used to determine total antioxidant capacity.

Changes in oxidative damage after physical exercise are directly related to the type, intensity, and duration of the exercise[25]. Thus, it has been reported that different exercises cause different levels of oxidative damage, although the results suggesting the influence of exercise on the levels of oxidative stress remain controversial. Increased production of ROS can oxidize membrane lipids causing lipid peroxidation[29]. During lipid peroxidation, there may be losses of intermediaries resulting in short-chain hydrocarbons (ethane, pentane), aldehydes (such as malonaldehyde, 4-hydroxynonenal), epoxides, and other highly cytotoxic prodcuts[9]. As a result of lipid peroxidation, membranes undergo changes in fluidity and permeability, resulting in a loss in homeostasis and cell death[9], leading to decreased performace[14].

Oxidation of amino acids causes formation of carbonyl groups and reduction of total thiols, among other changes that alter the normal function of proteins, being widely used as a marker of cell damage by ROS action[9]. The results of our study show an increase in protein carbonylation (PC) and reduction in the amount of total thiols (TTs). Several mechanisms may contribute to this phenomenon, such as increased mitochondrial electron flow, increased oxidation of purines, and imbalance in the calcium concentrations[29]. Therefore, the decrease in TTs can be explained by high levels of ROS produced during the Ironman race, since TTs are the first line of defense against the ROS attack[21]. Some studies[29,30] have shown similar results for changes in the markers of oxidative stress after prolonged exercise.

CONCLUSION

The results of the present study show clear evidence that long and highly intense races cause reduced antioxidant capacity and oxidative damage of athletes regardless of their performance during the race. Establishing a strategy of supplementation of antioxidants may help reduce these effects and improve the performance in long and exhaustive races.

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