Articulo original

Exhaustive physical exercise causes a decrease in oxidative stress and an increase in salivary total antioxidant activity of elite triathlete

El ejercicio físico extenuante causa una disminución del estrés oxidativo y un aumento de la actividad antioxidante total de la saliva del triatleta élite

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ABSTRACT

The aim of this research was to study the effect of exhaustive physical exercise (triathlon) on uric acid (UA), total antioxidant activity (TAA), lipid hydroperoxides and nitric oxide (NO) metabolite, nitrite, in the saliva of elite triathletes. Stimulated saliva was sampled from 12 elite triathletes, 1 h before and immediately after competition. UA was assayed by enzymatic method, TAA by ABTS method, lipid hydroperoxides by FOX method and nitrite concentration by the Griess reaction. Exhaustive exercise caused an increase in both salivary UA concentration and TAA immediately after competition. On the other hand, there was a decrease in salivary lipid hydroperoxides. These results suggested that exhaustive exercise causes a decrease in salivary lipid hydroperoxides, a marker of oxidative stress, and this result could be explained by the fact that both UA and TAA increased after the triathlon.

KEY WORDS

Nitric oxide, Uric acid, Exercise, Free radical, Oxidative stress, Antioxidant Activity, Nitrite, Triathlon.

RESUMEN

El objetivo de esta investigación fue estudiar el efecto del ejercicio físico exhaustivo (triatlón) en el ácido úrico (AU), la actividad del antioxidante total (AAT), hidroperóxidos lipídicos y en el metabolito del óxido nítrico (NO), el nitrito, en la saliva de triatletas élite. Se recolectó la saliva estimulada de 12 triatleta élite, 1 h antes e inmediatamente después de la competición. AU se ensayó por el método enzimático, AAT por el método de ABTS, los hidroperóxidos lipídicos por el método de FOX y la concentración del nitrito por la reacción de Griess. El ejercicio exhaustivo causó un aumento de la concentración de AU salival y AAT inmediatamente después de la competición. Por otro lado, hubo una disminución en los hidroperóxidos lipídicos de la saliva. Estos resultados sugieren que el ejercicio exhaustivo causa una disminución en los hidroperóxidos lipídicos salivales, un marcador del estrés oxidativo, y este resultado podría explicarse por el hecho que el AU y el AAT aumentaron después del triatlón.

PALABRAS CLAVE

Óxido Nítrico, Ácido Úrico, Ejercicio, Radical Libre, Estrés Oxidativo, Actividad Antioxidante, Nitrito, Triatlón.

INTRODUCTION

The production of free radicals and associated reactive oxygen species (ROS) increases markedly during sustained endurance exercise [1-3]. These ROS have the potential to trigger the cytotoxic process of lipid cell membrane peroxidation resulting in the formation of MDA (malondialdehyde) [4]. Antioxidant defenses may attenuate the deleterious effects of ROS and some free radical scavenging compounds such

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as uric acid have been found to rise in response to sustained endurance exercise [1,5]. It has been observed an increase in total antioxidant capacity following a marathon [1,6-9] that suggested an enhanced ability of organism to scavenge ROS in serum. However, the rise in serum TAA was insufficient to prevent exercise-induced lipid peroxidation evidenced by the concomitant elevation of MDA [1,5].

Recently, it has been demonstrated that aerobic exercise induced an increment in both salivary uric acid and TAA, and this increase seems to inhibit lipid hydroperoxide generation (a marker of oxidative stress) in human saliva [10]. These results suggested that saliva samples could be an excellent alternative tool for the study of effects of exercise on human organism. The noninvasive collection techniques of saliva samples dramatically reduce anxiety and discomfort and simplify procurement of repeated samples for monitoring over time.

Since saliva is the first important defense against oxygen toxicity in human. Therefore, the aim of the current study was to determine the effect of exhaustive exercise (triathlon) on uric acid, total antioxidant activity, oxidative stress and nitric oxide metabolites in the saliva of elite triathlete.

MATERIALS AND METHODS

Twelve elite triathletes (men, n = 7 and women, n = 5) were studied during the Falcon State-Venezuela 2006 Triathlon. Personal information and informed consent were obtained from each triathlete before competition. All procedures conformed to the code of Ethics of the World Medical Association (Declaration of Helsinki). The anthropometric characteristics of participants are present in Table 1.

TABLA 1
Subject anthropometric characteristics.

Age (years)	21.08 ± 0.38
Weight (kg)	59.33 ± 1.86
Height (m)	1.70 ± 0.02
Body Fat (%)	18.91 ± 0.78
Waist (cm)	77.16 ± 1.61
Hip (cm)	87.75 ± 1.49
BMI (kg/m^2)	20.42 ± 0.48
Competition time (min)	131 ± 2.87

Values are expressed as mean ± SEM.

Experimental procedure

After sufficient gargling with mineral water, stimulated whole saliva (5 mL) by chewing on paraffin was collected from each subject 1 h before and immediately after competition. Saliva samples were centrifuged at 3,000 rpm for 10 min and placed into Eppendorf tubes and stored in the refrigerator.

Uric acid (UA) determination

Uric acid concentration was measured in the saliva samples using a kit supplied by Qualitest (Industrias Qualitest, Venezuela) as previously described [11].

Total antioxidant activity (TAA) determination

The total antioxidant activity (TAA) of saliva samples in the reaction with stable 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was determined according to Re et al. [12] method with slight modifications. The $ABTS^{\cdot+}$ was produced by reacting ABTS with potassium persulfate ($K_2S_2O_8$). The ABTS was then dissolved in water to a 7 mM concentration. The ABTS radical cation was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) in the dark at room temperature for 12-16 h to allow the completion of radical generation. This solution was then diluted with 40 mM phosphate buffer (pH 7.4) so that its absorbance was adjusted to 0.600-0.700 at 734 nm. Ten microliters of saliva sample were mixed with 1 ml of $ABTS^{\cdot+}$ solution in the 1 cm path length microcuvette. The absorbance was read at room temperature after 0 and 6 min. The decolorization percentage of the absorbance at 734 nm was calculated by the formula I = [(Ab – Aa)/Ab] x 100; where; I = $ABTS^{\cdot+}$ inhibition, %; Ab = absorbance of a blank sample (t = 0 min); Aa = absorbance of a tested saliva sample at the end of the reaction (t = 6 min). TAA was calculated as mM (Trolox equivalents) from a calibration curve.

Ferrous ion oxidation with xylenol orange (FOX) method.

Lipid hydroperoxides were determined using the ferrous iron/xylenol orange (FOX) technique which incorporates the selective oxidation of ferrous to ferric ions by hydroperoxides [13]. Briefly, 900 µl of FOX's reagent (49 mg of ferrous ammonium sulfate in 50 ml of H_2SO_4 250 mM, 0.397 g BHT, and 0.038 g xylenol orange in 950 mL of HPLC grade methanol) was added to 100 µl of saliva sample and left to react for 30 minutes at room temperature. The absorbance was read at 560 nm. Hydrogen peroxide was used as standard.

Nitrite determination

The concentration of nitrite was determined by a colorimetric assay based on the Griess reaction [14]. Briefly, to 50 µL of saliva sample, 100 µL of 14 mM sulfanilamide in 2 N HCl, 100 µL of 4 mM N-(1-naphthyl)-ethylenediamine (NED) in water, and 750 µL of 0.2 M KCl-HCL (pH 1.5) were added. The samples were incubated at 37ºC for 10 min and then were centrifuged at 5,000 rpm for 10 min. Absorbance was measured at 540 nm and sodium nitrite was used as a standard.

Statistical analysis

Statistical analysis was carried out using a statistical package SPSS (Version 12.0 for Windows, SPSS Inc., Chicago). Results were expressed as Mean ± SEM. Paired t-test was used to examine the effect of exhaustive exercise on uric acid, TAA, lipid hydroperoxides and NO metabolites. Alpha was set a-priori at p = 0.05.

RESULTS

The results of this investigation showed a statistically significant effect of exhaustive exercise on salivary UA concentration. The pairwise comparison revealed that the UA concentration was higher immediately after competition (IAC) than at 1 h before competition (p = 0.02) (Fig. 1). Exhaustive physical exercise had also a statistically significant effect on salivary TAA. Indeed, the pairwise comparisons revealed that salivary TAA was higher IAC than at 1 h before competition (p = 0.0001) (Fig. 2).

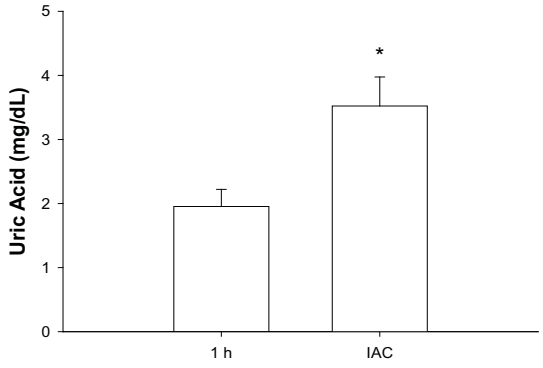

Figura 1. Effect of exhaustive exercise on salivary uric acid concentration. Conditions: 1 h: 1 h before exercise, IAC: Immediately after competition. Values were the means ± SEM. *, statistically significant difference from 1 h ($p < 0.02$).

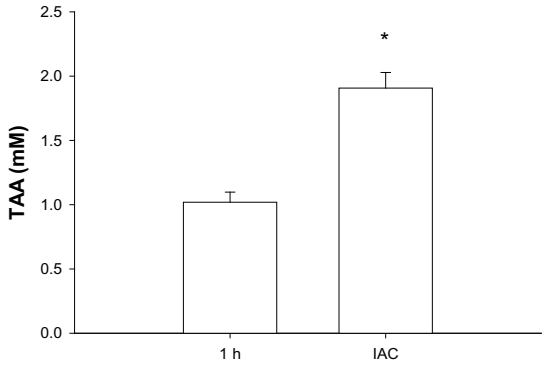

Figura 2. Effect of exhaustive exercise on salivary total antioxidant activity (TAA). Conditions: 1 h: 1 h before exercise, IAC: Immediately after competition. Values were the means ± SEM. *, statistically significant difference from 1 h ($p < 0.0001$).

On the other hand, exhaustive physical exercise caused a statistically significant effect on salivary lipid hydroperoxides (index of oxidative stress). Indeed, IAC salivary lipid hydroperoxides were lower than at 1 h before competition (p = 0.03) (Fig. 3). Exhaustive exercise did not have effect on salivary nitrite concentration (result not shown).

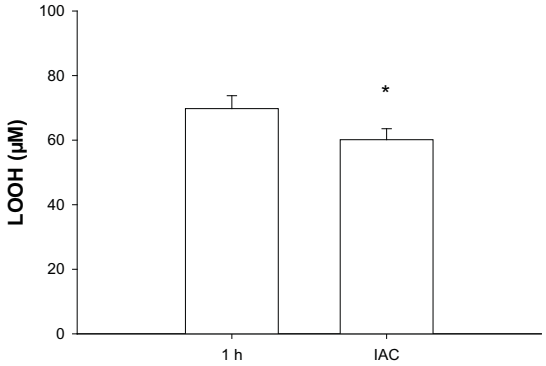

Figura 3. Effect of exhaustive exercise on salivary lipid hydroperoxides (LOOH). Conditions: 1 h: 1 h before exercise, IAC: Immediately after competition. Values were the means ± SEM. *, statistically significant difference from 1 h ($p < 0.03$).

DISCUSSION

In this investigation, it was demonstrated that exhaustive physical exercise induced a statistically significant increase in salivary UA concentration. UA is an end product of purine metabolism and has been suggested to function as the most important antioxidant molecule in saliva [15-18]. Our results indicated that salivary UA increased in response to

exhaustive exercise and these results were consistent with the findings of others in saliva [10,19] and in plasma [2,5,20-23]. Some authors have suggested that the exercise-induced increase in salivary UA could be due to the enhanced purine oxidation and subsequent formation of UA [5,20,21,24].

Exhaustive exercise also caused a significant increase in salivary TAA which may reflect enhanced antioxidant defences in response to the exercise-induced oxidative stress. Moreover, our findings were in accordance with previous studies in plasma reporting increased antioxidant nutrients and antioxidant potential in response to extreme exercise [1,5-9,25]. There are very few reports on the relationship between salivary TAA and exercise, only, González et al. [10] also reported an increase in salivary TAA after a 10,000-m race. The increase in both UA and TAA could be explained by a correlation between these two parameters as has been suggested by other authors [26,27]. Moore et al. [16] reported that the TAA of saliva correlated with the concentration of UA, which contributes more than 70% of the TAA.

As a result of exhaustive exercise, there was a decrease in oxidative stress immediately after competition. To the best of our knowledge, our work is the first report about lipid hydroperoxide status in the oral compartment after exhaustive exercise (triathlon). González et al. [10] found that after a 10,000-m race salivary lipid hydroperoxide decreased as well.

Nitric oxide metabolites in human body fluids, nitrite and nitrate, have been used as indicators of oxidative and nitrosative stress [28-30]. Our results did not show any effect of exhaustive exercise on salivary nitrite concentration, this could be explained by the scavenging action of UA on reactive nitrogen species as has been suggested by several authors [31-33]. González et al. [10] also reported that after a 10,000-m race there was no change in salivary nitrite concentration. Interestingly, Sureda et al. [34] showed that the nitrite levels in venous plasma and blood cells after 3 h of intense exercise were fairly similar to the basal ones. They suggested that apparently nitrite plasma concentration is regulated to maintain constant plasma levels and the metabolic pathways that tightly regulate circulating nitrite are not well elucidated but were operative during exhaustive exercise. Alessio et al [35] also showed that there was no difference in NO when animals were exercised to exhaustion. Palomero et al. [36] also demonstrated that passive stretching applied to single mature skeletal muscle fibers did not induce a significant increase in the generation of intracellular NO. Monteiro et al. [37] concluded that intense exercise did not increase NO bioavailability. However, Panossian et al. [38] demonstrated an increase in salivary NO after heavy physical exercise, and treatment with adaptogens inhibited this increase in athletes.

CONCLUSIONS

In conclusion, this data suggests that exhaustive exercise-induced increment in both TAA and UA tended to reduce exercise-induced oxidative stress in human saliva. Salivary analysis, which is less invasive and much easier to perform as compared with plasma analysis, is suggested as a new and effective tool in exercise studies.

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