Chemico-Biological Interactions 227 (2015) 89–95

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Effects of chronic resveratrol supplementation in military firefighters undergo a physical fitness test – A placebo-controlled, double blind study

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ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 14 December 2014
Accepted 19 December 2014
Available online 5 January 2015

Keywords:
Resveratrol
Inflammation
Exercise
Oxidative stress
Polyphenols

ABSTRACT

Purpose: The purpose of this study was to determine the plasma metabolic response and certain indicators of oxidative stress (antioxidant system and oxidative stress biomarkers) in plasma and erythrocytes of Brazilian military firefighters supplemented or not with resveratrol (RES) for 90 days (100 mg/day). The analyses were performed before and after a typical physical fitness test (FT) used to induce oxidative stress.

Methods/Results: In this placebo-controlled double-blinded study, we observed that RES supplementation did not present hepatic consequences compared with the placebo group following analysis of AST, ALT and GGT plasma activities. Plasma glucose and triglycerides levels were increased after the FT in firefighters supplemented with RES but were not elevated at baseline. Neither total nor cholesterol fractions were modified by RES supplementation. CK levels were increased after the firefighters performed the FT; however, no differences were determined between the placebo and RES groups. Ferric-reducing ability of plasma as well as uric acid was increased after the FT, but was not modified by RES supplementation. Plasma oxidative stress biomarkers, such as thiol content, 8-isoprostane and 8OHdG, showed no modifications, while IL-6 and TNF-α were decreased in the RES group after the FT. Among antioxidant enzyme activities determined in erythrocytes from the firefighters, only GPx activity was reduced by RES supplementation both before and after the FT.

Conclusion: In summary, the most pronounced effect of RES supplementation is its anti-inflammatory effect, which reduced IL-6 and TNF-α level. The FT applied to Brazilian military firefighters was not sufficient to challenge the antioxidant defense systems, and, therefore, 100 mg of RES for three months did not induce significant effects.

1. Introduction

Over the past 20 years, interest in the benefits of natural polyphenolic compounds has increased. The polyphenolic compound resveratrol (RES), 3,5,4′-trihydroxy-trans-stilbene, is a molecule which consists of two aromatic rings attached by a methylene bridge that is naturally synthesized by several plants in response to adverse conditions, such as environmental stress, or when under attack by pathogens, such as bacteria or fungi. It is especially present in grapes, peanuts, cranberries, blueberries and mulberries and the *trans* isomer is found naturally far more than the *cis* isomer, even though only trace amounts are observed, and these contribute minimally to the diet [1,2]. RES has gained attention because it is found in red wine, which is associated with the "French Paradox". French people normally consume more saturated fat in their diet and still present lower incidence of coronary heart disease; this has been attributed to the high consumption of red wine, which contains high levels of RES [3].

The physiological properties of RES include anti-inflammatory, anticancer, anti-aging, antioxidant and antidegenerative actions [4,5]. The anti-inflammatory effects of RES have been demonstrated in several animal studies [6–8]. More recently, RES treatment has been shown to be beneficial for suppressing the aging-related decline in physical performance [9], reducing functional decrements and oxidative stress levels in rat hind limb muscles in response to disuse [10] and optimizing fatty acid

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http://dx.doi.org/10.1016/j.cbi.2014.12.033
0009-2797/

metabolism, which may contribute to the increased contractile force response of skeletal muscles and improved parameters of cardiac structure and function in rats submitted to physical activity [11]. RES exerts its various biological actions on a variety of targets, including the cell surface and some intracellular receptors, the molecules of signal transduction, metabolism, DNA repair proteins and transcription factors [12].

The regular practice of physical activity is an important factor for health promotion. However, frequent high intensity or exhaustive physical exercise can increase susceptibility to injury, promote chronic fatigue and overtraining, partially due to the overproduction of reactive oxygen species (ROS). It is clear that aerobic and anaerobic exercises potentially stimulate ROS production, which can lead to a state of oxidative stress. Different exercise protocols can produce a variety of stimuli for ROS production, as shown by oxidative stress biomarkers that are dependent on exercise intensity and duration [13–15]. In low-intensity exercise protocols, antioxidant defenses appear to be sufficient to combat the increase in ROS production with no evidence of oxidative damage. In addition, ROS are central player's by acting as signaling molecules in several cellular pathways involved in adaptations to exercise [16] Numerous studies have investigated a variety of antioxidant supplements in an effort to mitigate and/or attenuate oxidative damage induced by exercise and modulate the enzymatic antioxidant defenses [17].

The goal of physical training of firefighters is to induce physiological adaptations thus increase physical performance. To achieve this goal, it is common apply a variety of programs and physical training methods based on principles like overload, specificity and progression of training that are often accompanied by an improvement in overall fitness. The work of military firefighters includes complex actions subdivided into different tasks that require substantial levels of fitness in strength, power, aerobic and muscular resistance, as well as psychological balance. Moreover, during the preparation stages of Brazilian military firefighters, they are periodically challenged with a typical physical fitness test (FT) [18], performed in order to verify improvement in the fitness of these professionals. Thus it is reasonable to assume that military firefighters may be exposed to a condition of oxidative stress due to the frequency and intensity of physical activities typical of their daily tasks. We hypothesized that the FT applied periodically to the firefighters can increase ROS production and cause significant oxidative stress in plasma and erythrocytes of these subjects and that supplementation with RES can restore the changes induced by the FT. In parallel, the firefighters can show an improvement in the performance due to RES supplementation. The purpose of this study was to determine the plasma metabolic response and certain indicators of oxidative stress (antioxidant system and oxidative stress biomarkers) in plasma and erythrocytes of Brazilian military firefighters supplemented or not with RES for 90 days (100 mg/day). The analyses were performed before and after a typical physical fitness test, supposedly an inducer of oxidative stress.

2. Materials and methods

2.1. Participants

A placebo-controlled, triple-blind study was conducted on 60 out of 250 eligible military firefighters, who were divided in two groups (RES and placebo, $n = 30$ each). All 60 male military firefighters from the Soldiers Training Course of the School Military Police Firefighters of São Paulo, Brazil, volunteered to participate in the study over the course of the 2012 preparative stage (Table 1). They were informed about the experimental procedures and possible discomforts associated with the study before providing written informed consent of their participation. The experimental procedures and protocol conformed to the principles of the Declaration of Helsinki and were approved by the Human Research Ethics Committee of Cruzeiro do Sul University, under protocol No. CE/UCS 133/2011.

Table 1
Anthropometric characteristics of the military firefighters.

	Placebo (mean ± SEM)	Resveratrol (mean ± SEM)
Age (year)	22.3 ± 1.78	21.46 ± 1.77
Height (cm)	175.6 ± 0.6	174.7 ± 0.5
Body mass (kg)	67.78 ± 6.51	69.48 ± 6.42
Body fat (%)	9.97 ± 1.89	9.00 ± 2.54
Heart rate (beats per min)	68.73 ± 10.95	70.13 ± 10.54
Blood pressure (mmHg)	120/81 ± 10/5	121/81 ± 10/5

2.2. Study products and intervention

The study products were provided to military firefighters in identical code labeled (RES) capsules containing 100 mg of either maltodextrin (placebo) or RES and packed in the same code labeled blank boxes. The polyphenolic composition of the RES capsules was ⩾98% trans-resveratrol derived from *Polygonum cuspidatum* kindly provided by Farmel Pharmacy (São Paulo, SP, Brazil). The participants were instructed to take 1 capsule/day in the morning for 3 months until conclusion of the trial. Moreover, the participants were requested to maintain their customary lifestyle and diet throughout the trial and to minimize the intake of fatty meals, antioxidant supplements and grape-derived products, including red wine. Dietary habit changes, treatment interruptions and adverse effects (digestive discomforts, allergic reactions, etc.) were monitored through questionnaires and phone calls throughout the study. In addition, the participants noted down their dietary intake in the three days prior to blood withdrawal. Total energy intake was 2800 kcal/day.

2.3. Study design

Blood samples were collected in the morning between 8 and 11 am, following the ingestion of their first meal (breakfast). Blood collection was performed by a team composed of qualified medical doctors and nurses immediately before and after completion of the fitness test (FT). To minimize possible changes in biochemical analysis, blood sampling was performed in stages, totaling a week of collection (staggering 12 participants per day). It should be emphasized that the FT was also scheduled to allow for blood collection under the same conditions for all participants. The physical FT is a protocol used by the Military Police of São Paulo, consisting of four (04) exercises performed in sequence: (1) chin-up, (2) abdominal sit-up, (3) speed test: 50 meters sprint; (4) aerobic exercise: running for 12 min (Cooper test). The firefighters must perform as many chin-up and abdominal sit-up as possible in 1 min, run the 50-meter sprint in the shortest possible time and run the maximum distance in the 12-min time trial.

Thus blood was collected at four different moments: T1, before supplementation and before the FT; T2, before supplementation and after the FT; T3, following supplementation (RES or placebo) and before the FT; T4, following supplementation and after the FT (Fig. 1). A total of 240 biological samples (120 from the resveratrol group and 120 from the placebo group) were collected from the participants and composed the data in this study. Prior to T1 and between T2 and T3 periods (90 days of RES supplementation), the subjects were conducting 2 h/daily of regular exercise training of moderate intensity, without performing the FT.