Nutrition 32 (2016) 539–545

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Effects of pomegranate juice in circulating parameters, cytokines, and oxidative stress markers in endurance-based athletes: A randomized controlled trial

E. Fuster-Muñoz Ph.D.(c) [a], E. Roche Ph.D. [b], L. Funes Ph.D. [c], P. Martínez-Peinado Ph.D.(c) [d], J.M. Sempere Ph.D. [d], N. Vicente-Salar Ph.D. [b,*]

[a] Toxicology Unit, Institute of Bioengineering, University Miguel Hernandez, Elche (Alicante), Spain
[b] Biochemistry and Cell Therapy Unit, Institute of Bioengineering, University Miguel Hernandez, Elche (Alicante), Spain
[c] Quality and Innovation Department, Vitalgrana Pomegranate SL. Polígono Industrial de Poniente, Catral (Alicante), Spain
[d] Immunology Division, Biotechnology Department, University of Alicante, San Vicente del Raspeig (Alicant), Spain

ARTICLE INFO

Article history:
Received 23 May 2015
Accepted 5 November 2015

Keywords:
Pomegranate juice
Antioxidants
Sport
Carbonyls
Malondialdehyde

ABSTRACT

Objective: The aim of the present study was to assess the effects of pomegranate juice on the level of oxidative stress in the blood of endurance-based athletes. Pomegranate juice is rich in polyphenols, conferring it a higher antioxidant capacity than other beverages with polyphenolic antioxidants.

Methods: A randomized double-blind, multicenter trial was performed in athletes from three different sport clubs located in southeastern of Spain. Plasma oxidative stress markers (protein carbonyls and malondialdehyde [MDA]) as well as C-reactive protein and sE-selectin were measured. Thirty-one athletes participated in the study. Participants were divided into three groups. The first group was supplemented with 200 mL/d pomegranate juice (PJ; n = 10) over a 21-d period, the second with 200 mL/d pomegranate juice diluted 1:1 with water (PJD; n = 11), and a control group that did not consume pomegranate juice (C; n = 10). Nine athletes were excluded due to protocol violations (n = 4 in the PJ group and n = 5 in the PJD group) because they did not observe the 24 h of rest before the last blood test.

Results: The control group increased levels of carbonyls (+0.7 ± 0.3 nmols/mg protein) and MDA (+3.2 ± 1.0 nmols/g protein), whereas the PJ and PJD groups maintained or decreased their levels, respectively. On the other hand, lactate levels increased in the PJ group (from 10.3 at day 0 to 21.2 mg/dL at day 22). A nonsignificant decrease was detected in sE-selectin and C-reactive protein in the groups consuming pomegranate juice.

Conclusion: Consumption of pomegranate juice over a 21-d period improved MDA levels and carbonyls, and thus decreased the oxidative damage caused by exercise.

Introduction

Reactive oxygen and nitrogen species (RONS) play various roles in the cells, both beneficial and deleterious. The beneficial effects of RONS include defense against infectious agents as well as intracellular signaling molecules in many processes [1]. However, persistently high RONS levels can produce harmful effects if the antioxidant defenses are overwhelmed, resulting in

This research received specific grant and provision of supplements from Vitalgrana SL. Vitalgrana SL had no role in the design, analysis or writing of this article. ER has received honoraria from Vitalgrana SL and L. Funes is on the speakers' bureau for Vitalgrana SL. EF-M was responsible for the anthropometric evaluation, biochemical determinations, and analysis of results. ER was responsible for the study design and writing the article. LF was responsible for supplement production and PM-P for cytokine determinations. JMC was responsible for cytokine determinations and writing the article. NV-S was responsible for diet control and design, contact with participants, study design, analysis of results, and writing the article. The authors acknowledge the technical support of José María Adsuar and the participants of this study. The following institutions are acknowledged PROMETEO/2012/007 from Generalitat Valenciana and CIBEROBN (Fisiopatología de la Obesidad y la Nutrición CB12/03/30038) Instituto de Salud Carlos III, Spain to E Roche.

* Corresponding author. Tel.: +34 965 22 2041; fax: +34 965 91 9546.
E-mail address: Nvicente@umh.es (N. Vicente-Salar).

http://dx.doi.org/10.1016/j.nut.2015.11.002
0899-9007/

structural damage, including membrane lipids, proteins, and nucleic acids. This phenomenon is called *oxidative stress*, and can be detected by analyzing end metabolites of the oxidation process such as malondialdehyde (MDA) from lipid peroxidation in the blood, or by measuring oxidatively altered macromolecules such as the presence of carbonyl adducts in affected proteins [2–4].

Exercise could be considered an exogenous source of oxidative stress due to an increase in oxygen consumption at the level of mitochondrial respiration, leading to punctual increases in RONS production [5]. However, this is a controversial topic because the benefits of exercise are well documented in the prevention or treatment of chronic disorders such as diabetes mellitus; dyslipidemia; hypertension; obesity; cardiovascular and pulmonary diseases; muscle, bone, and joint diseases; cancer; and depression [6]. After moderately intense exercise, the muscle tissues produce RONS, which have been shown to act as intracellular secondary messengers [7]. However, when strenuous exercise or overloaded training is performed, an imbalance occurs between production of free radicals and the endogenous antioxidant systems [8–10]. Moreover, high levels of markers of oxidative stress and inflammation, such as E-selectin and C-reactive protein (CRP), could lead to endothelial dysfunction [11,12].

Nevertheless, diet is the main source of antioxidants and in this context, pomegranate juice, which is extracted from the fruit of the *Punica granatum* plant, is rich in polyphenols such as anthocyanins, flavonols, and certain ellagitannins such as punicalagin [13]. Several studies have documented the benefits of pomegranate juice consumption in individuals affected with various disorders [14–20]. Regarding the field of physical activity, only a few studies have analyzed how pomegranate consumption can modulate performance during exercise [21,22]. However, there are no studies regarding the possible role of pomegranate juice consumption in oxidative stress modulation in athletes. Thus, the aim of the present study was to assess the effects of pomegranate juice on oxidative stress markers in a group of well-trained endurance-based athletes.

Material and methods

Trial design

Participants were randomly assigned to one of three groups that consumed the juice on both training and nontraining days. On training days, juice was consumed immediately after the training session as a postexercise meal: Those who consumed a 200-mL bottle of pomegranate juice daily (PJ group; n = 10), another group that consumed a 200-mL bottle of pomegranate juice diluted 1:1 with water daily (PJD group; n = 11), and a control group that consumed one piece (~200 g) of seasonal fruit other than pomegranate, which contained the same energy as one 200-mL bottle of pomegranate juice, instead of pomegranate juice (C group; n = 8) for maintaining the same daily energetic intake (Table 1). The aim of the PJD group was to determine whether there was a dose response in any of parameters studied. All the groups consumed fluids as water after exercise ad libitum. The design was a double-blind, parallel-group, randomized controlled trial conducted at Miguel Hernandez University of Elche (Spain).

Participants

Volunteers participating in the study (Table 1) were selected from sport clubs in various locations in southeastern Spain. The parameters for inclusion were to be adult men, perform training sessions, and to have participated recently in a half marathon or similar event, which are held throughout the competitive season. To this end, the participants performed endurance-based training for more than 1 h per session and more than three sessions per week. The exclusion criteria were intake of antioxidant or anti-inflammatory supplementation, presence of a chronic disorder, smoking, and consuming alcoholic beverages.

Table 1
Anthropometric values of each group at day 0 (d0) and day 22 (d22)

Group	C (n = 8)		PJ (n = 6)		PJD (n = 6)	
	Mean	SD	Mean	SD	Mean	SD
Age (y)	33.3	9.0	35.2	8.5	37.5	11.4
Height (m)	1.7	0.1	1.7	0.1	1.7	0.1
Weight (kg) (d0)	71.3	11.8	67.2	3.4	70.0	12.2
Weight (kg) (d22)	70.3	11.7	66.8	3.8	70.1	12.1
%Fat mass (d0)	14.2	4.4	15.7	6.0	16.3	5.4
%Fat mass (d22)	13.1	4.2	14.5	5.2	15.7	4.8
%Muscle mass (d0)	46.1	4.7	46.4	4.0	43.9	5.3
%Muscle mass (d22)	46.3	4.6	46.7	4.2	43.3	4.8

C, control group; PJ, group consuming pomegranate juice; PJD, group consuming pomegranate juice diluted 50% with water

Interventions

Thirty-one volunteers were selected (Fig. 1), informed about the objective and demands of the study, and gave their written consent to participate. The protocol was in accordance with local legal requirements and the Helsinki Declaration for research on human beings, and approved by the Ethics Committee of University Miguel Hernandez.

Pomegranate juice was provided by Vitalgrana SL (vitalGrana, Alicante, Spain), elaborated by crushing the fruit and the seed. This produces the transfer of an oily phase to the final juice that is rich in unsaturated fatty acids, with punicic acid being one of the most abundant. (The complete composition of the juice can be found at http://www.vitalgrana.com/es/productos-zumo-granada.) Study duration was 5 wk (Fig. 1). During the first 2 wk of the experiment (homogenization period), participants started their training sessions and verified accomplishment of the duration and frequency. This period was used to allow participants to become accustomed to the individual diet plan and to resolve doubts about the procedure. During the homogenization period, nine participants were excluded due to protocol violations in the training program (Fig. 1). The recruitment process began in August 2012, and the intervention was carried out in February 2013.

Diet plans were customized, adjusting energy expenditures and macronutrients to the training activity and to each athlete's body weight (Table 2), so that diet composition or energy intake did not affect the study. Total energy expenditure (TEE) was estimated as an average of the resting energy expenditure (REE) for each weight range according to the Food and Agricultural Organization equation ([11.3 × weight] + [16 × {height/100}] + 901) [23] and multiplied by 1.55 as a factor for activity. Therefore, all groups had the same diet plan according to individual weight, with the only exception the substitution of one portion of fruit in group C by juice in groups PJ and PJD to maintain the energy intake. Participants were instructed to manage their own diet plan by making proper equivalent food changes (maintaining the daily energy intake and macronutrient composition) during the 3-wk intervention. Periodic meetings were maintained to resolve any problems the participants had during the study. The next 3 wk were considered the intervention period. This was when data was collected, and coincided with other studies that used similar periods of time [14,24]. At day 0, before starting the intervention and after 48 h without exercise, whole blood samples were collected and anthropometric measures performed according to recommendations of the International Society for Advancement of Kinanthropometry (ISAK). At the end of the study (day 22), volunteers repeated the aforementioned procedures. The participants scored their physical activity and its duration during the 22 d of intervention. The energy expenditure by exercise of each athlete was calculated through metabolic equivalent values of each activity and shown as the main of energy consumed per day (Table 1).

Blood samples were obtained from the antecubital vein in EDTA vacutainers at days 0 and 22 after overnight fasting. Plasma was obtained as a supernatant of the whole blood after centrifugation at 1000*g* for 15 min at 4°C and then stored at −80°C.

Circulating glucose was determined by the glucose oxidase method coupled with the peroxidase reaction [25]. High-density lipoprotein cholesterol (HDL-C) was determined by a direct enzymatic colorimetric method. HDL was dissolved with a detergent, whereas HDL-C was released to react with cholesterol esterase. Afterward, free cholesterol was oxidized with cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide, which was determined using the peroxidase reaction. The non-HDLs were inhibited from reacting with the enzymes due to the absorption to the detergent [26]. Circulating triacylglycerols were determined from coupled reactions of lipoprotein-lipase, glycerol-kinase, glycerol phosphate oxidase, and peroxidase, giving a color end-adduct as reported previously [27]. Ferritin was determined using an enzyme-linked fluorescent assay (BioMerieux, Madrid, Spain) according to the manufacturer's instructions. Lactate was determined by lactate oxidase/peroxidase-coupled colorimetric