Thrombosis Research 135 (2015) 255–259

Contents lists available at ScienceDirect

Thrombosis Research

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

Regular Article

Orange juice intake during a fatty meal consumption reduces the postprandial low-grade inflammatory response in healthy subjects

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ARTICLE INFO

Article history:
Received 9 July 2014
Received in revised form 16 October 2014
Accepted 4 November 2014
Available online 13 December 2014

Keywords:
Fatty meal
Orange juice
Vascular function
Low-grade inflammation
Myeloperoxidase

ABSTRACT

Background and Aims: Evidence associates polyphenol-rich foods to reduction of low-grade inflammation and mortality for cardiovascular disease, the mechanisms underlying such effects being still unclear. Consumption of a fatty meal by healthy volunteers induces rapid and reversible low-grade inflammation. The aim of the present study was to evaluate the effect of orange juice on cellular modifications induced by a fatty meal.
Methods and Results: 18 apparently healthy subjects consumed a fatty meal, during which they drunk orange juice, either blond or red, or water, according to a randomized cross-over design. Two hours after the end of the fatty meal, both white blood cell (WBC) and platelet counts significantly increased (12.5 and 5 %, respectively), while mean platelet volume decreased and a 25% release of myeloperoxidase (MPO) from polymorphonuclear leukocyte occurred. Both juices significantly prevented WBC increase and MPO degranulation, in respect to control. Triglycerides significantly increased (42%) after the fatty meal, but at a lower extent when red orange juice was consumed with the meal (20%), in respect to blond orange juice or control. This effect was statistically significant in the subgroup of 8 subjects with hypertriglyceridemia. Vascular stiffness (augmentation index), measured by Endo-PAT2000, significantly decreased after the meal only in conjunction with red orange juice.
Conclusion: In healthy subjects the concomitant intake of orange juice may prevent the low-grade inflammatory reaction induced by a fatty meal, at cellular and possibly at vascular function levels. The relative role of different polyphenols on the observed effects of orange juices remains to be established.

Introduction

The consumption of a fatty meal by healthy volunteers induces rapid changes in the number and activation status of circulating cells, in particular white blood cells, possibly due to the postprandial acute metabolic changes, including triglyceride increase [1]. Some of the determinants of the cellular response to a fatty meal have been characterized suggesting that a fatty meal is a test of oxidative stress or acute in vivo low grade inflammatory reaction [2]. This test can be of interest for the evaluation of the potential antioxidant effect of anti-inflammatory agents or nutrients [2].

Increasing scientific evidence associates the Mediterranean diet, and in particular the consumption of polyphenol-rich foods or beverages mainly of plant origin, with a reduction of low-grade inflammation and mortality for cardiovascular and neurodegenerative disease, although the molecular mechanisms underlying such an effect are still to be clarified [3,4]. In a recent cross-sectional analysis of the Molisani population, two cellular biomarkers of chronic low-grade inflammation, platelet and white blood cell counts, were found to be inversely related to the adherence to a Mediterranean diet, suggesting a role of consumption of dietary antioxidants on these parameters [5].

Epidemiological and clinical data support the association of leukocyte count with the occurrence of cardio- and cerebro-vascular disease [6,7]. A higher leukocyte count is associated with cardiovascular risk in healthy subjects, or may represent a worse prognostic factor in cardiovascular patients undergoing vascular intervention [8–10]. In addition, activation of circulating inflammatory cells, such as polymorphonuclear (PMN) leukocytes and monocytes, membrane expression of adhesion molecules involved in their interaction with platelets and endothelial cells and the release of oxidation products may play a pathological role in the development of atherogenesis and thrombosis [11]. Moreover, lipoproteins have been shown to induce the release of bioactive compounds, such as cytokines, nitric oxide and reactive oxidative species, affecting cell systems of the vascular wall, blood pressure and hemostasis [12].

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http://dx.doi.org/10.1016/j.thromres.2014.11.038
0049-3848/

The aim of this study was to evaluate in healthy volunteers the effect of two types of orange juice, either poor or rich in anthocyanins (a largely represented class of polyphenols, responsible for the blue, red and purple colors of many fruits, vegetables and grains present in the Mediterranean diet) on the modifications induced by a fatty meal on peripheral blood cells and on the endothelial function.

Materials and Methods

Study Subjects

Eighteen healthy volunteers (36.9 ± 10.5 years old; nine women) participated in the study; they were recruited as clinically healthy subjects bearing at least one cardiovascular risk factor (overweight or obesity, hypertension, smoking, high serum cholesterol or triglyceride levels). They were not receiving any chronic or occasional pharmacologic treatment during the study period. Exclusion criteria were treatment with anti-lipidemic drugs, previous cardiovascular events, pregnancy/lactation, alcohol abuse, gastrointestinal or cardiovascular diseases, type 2 diabetes, metabolic and endocrine diseases, vegetarian or other restrictive dietary requirements. Each subject underwent a clinical screening (body weight, blood pressure/pulse, lipids, liver and renal function, personal history and a dietary questionnaire) before being enrolled in the study. The study protocol was approved by the Ethical Committee of the Catholic University (Rome) and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All volunteers gave their informed written consent before starting the experiments.

Experimental Design, Fatty Meal And Orange Juices

After a three day run-in period to further assess participant's eligibility and commitment to the study, the subjects were assigned the sequence of treatments, according to a randomized cross-over design (with six different treatment sequences), and were invited to follow their usual dietary habits and maintain their current lifestyles.

Venous blood was collected in the morning with minimal venous stasis on overnight fasting subjects; after then, all subjects consumed within 15 minutes a freshly prepared standardized test meal [1], composed of two scrambled eggs, 3 slices of white bread (51 g), 30 g butter, 30 g bacon fried in butter. The nutrient composition was 25 g protein, 81 g carbohydrate, and 52 g lipid, for a total of 890 kcal. Blood collection was repeated two hours after the fatty meal consumption (a time selected on the basis of a preliminary study as long enough to induce a significant elevation of triglyceride plasma levels, while minimizing any possible circadian effect).

The intervention consisted in the intake during the fatty meal of one liter of either red (anthocyanin-rich) or blond (anthocyanin-poor) orange juice, or water (control). The time interval between each treatment was 7 ± 2 days. Red juice was obtained from Moro, Tarocco and Sanguinello orange varieties, while blond juice derived from Valencia, Navel and Belladonna orange varieties, properly selected by the Istituto Sperimentale per l'Agrumicultura (Acireale, Italy) and supplied by Ortogel (Caltagirone, Italy). Both juices, pasteurized and stored a 4 °C until use, were characterized by the same nutritional properties and phenolic composition with the exception of total anthocyanins that were undetectable in blond juice but averaged 53.1 ± 5.3 mg/L in the red one [13].

Biochemical Measurements And Blood Cell Counts

Biochemical analyses were done on fresh samples: serum lipids and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab350, Instrumentation Laboratory, Milan, Italy); blood cell counts, including leukocyte subpopulations, were measured in EDTA-K tubes using an automated haematology analyzer (Beckman Coulter HmX, Instrumentation Laboratory (IL), Milan, Italy).

Cellular Activation Biomarkers

Markers of cell activation (platelet P-selectin, leukocyte Mac-1 expression and myeloperoxidase (MPO) content), and interaction (mixed platelet-leukocyte conjugates) were measured by flow cytometry in citrated whole blood, as previously described [1,13,14]. In particular, for intracellular MPO detection in polymorphonuclear (PMN) leukocytes, the FIX & PERM® kit was used to allow anti-MPO antibody cellular access, as specified by the manufacturer (Invitrogen, Walter Occhiena, Torino, Italy). After 20 min incubation in the dark at room temperature,,red cells were lysed, supernatant discarded and resuspended samples acquired at flow cytometer (Coulter EPICS XL Beckman Coulter). For each measurement, 10,000 events were analyzed. Platelets and leukocytes were identified by morphological and immunological characteristics, using forward light scatter (FS) *vs*. side light scatter (SS) intensity. IOTest 3 lysing solution and monoclonal antibodies for flow cytometry (and their isotypic controls) were from Instrumentation Laboratory (Milan, Italy). Data are reported as percentage of cells positive for the specific antigen, including intracellular MPO.

Blood Pressure And Arterial Reactivity Measurements

Blood pressure (BP) and heart rate were measured by an automatic device (OMRON-HEM-705CP), according to the European Society of Hypertension recommendations [15]. A non invasive assessment of the endothelial function was performed before and three hours after the fatty meal by using the Endo-PAT2000 system (Itamar Medical's, Caesarea, Israel) [16,17]. The test was performed with the patient supine in a comfortable, thermo-neutral environment. Probes were placed on the forefinger of both hands. Continuous recording of pulsatile blood volume responses from both hands was initiated. After a 10-min equilibration period, a blood pressure cuff on the study arm was inflated to 60 mm Hg above systolic pressure and anyway to at least 200 mmHg for 5 min. The cuff was then deflated to induce reactive hyperaemia (RH), whereas peripheral arterial tonometry (PAT) recording continued for at least 5 min. The RH index was calculated as the ratio of the average amplitude of the pulse wave signal over a 1-min time interval starting 1 min after cuff deflation divided by the average amplitude of the signal of a 3.5-min time period before cuff inflation, normalized to the control arm to compensate for potential systemic changes. The Framingham RH index (RHI) was used [18]. The augmentation index (AI), a measure of arterial stiffness, was computed as the difference between the first and second systolic peaks of the central pressure waveform, expressed as a percentage of the pulse pressure, normalized to a heart rate of 75 per min [19].

Statistical Analyses

Calculation of the required volunteer sample size was based on an expected 10% reduction by juice ingestion of the biochemical and cellular changes induced by the fatty meal. Results are given as means ± SD. Data were first analyzed by a paired-samples *t* test to determine significant differences for each measurement performed before (T0) and after test meal (T1) (alpha level was 0.05). To determine significant differences between treatments (water, blond and red orange juice) of the delta (T1-T0) values for each parameter considered, pairwise contrasts were adjusted following the Sidak method, as outlined by Ludbrook [20]. SPSS software package for Windows (version 15.0) was used to perform the analyses.

Results

Table 1 reports the mean values of the risk factors of the participants and the number of subjects whose factor levels exceeded the cut-off.