Food Research International xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

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

The effect of raisins on biomarkers of endothelial function and oxidant damage; an open-label and randomized controlled intervention

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ARTICLE INFO

Keywords:
Oxidative stress
Endothelial function
Raisins
FMD
PWV
Antioxidants

ABSTRACT

Based on the existing data in grapes and wine, the aim of the present study was to investigate the probability that raisins improve clinical features and markers of oxidative stress, inflammation and arterial function in healthy smokers. Thirty-six apparently healthy smokers were recruited to an open-label and randomized, controlled, 4-week prospective intervention. All participants were reported to consume less than the recommended amount of five servings fruits and vegetables daily. Participants in the intervention were instructed to consume raisins equal to five fruit servings (90 g/d). Anthropometric and blood pressure (BP) measurements, assessment of dietary intake, and fasting blood draws were conducted at baseline and at week 4. Biochemical (glucose, lipids, liver enzymes), inflammation [C-reactive protein (CRP), leptin], oxidative stress [Malondialdehyde (MDA), Advanced oxidation protein products (AOPPs)] and arterial function markers [Flow-mediated dilatation (FMD), Pulse wave velocity (PWV), Intercellular adhesion molecule-1 (ICAM-1), Nitric oxide (NO)] were assessed pre- and post-intervention. Baseline characteristics did not differ between the intervention and control arm. No effect of daily raisin consumption was observed on markers assessed between baseline and week 4 in either arm. Regarding vegetable consumption, no difference was observed in either group between baseline and post-intervention; however, as expected, a significant increase was reported in the intervention arm in fruit consumption between baseline and end point ($p < 0.001$) and between two arms post-intervention ($p < 0.001$). When analyzing according to age, ICAM-1 levels significantly decreased in subjects > 30 years ($n = 8$) in intervention arm (390.1 ± 17.6 to 302.2 ± 11 ng/mL, $p = 0.004$). After analysis of the data for sex, women in intervention ($n = 5$) decreased significantly diastolic BP (74.6 ± 4.2 to 67.4 ± 2.6 mg/dL, $p = 0.043$), total cholesterol (175.8 ± 7 to 166.6 ± 6.6 mg/dL, $p < 0.001$) and LDL-cholesterol (96.2 ± 9.6 to 89 ± 10.5 mg/dL, $p = 0.012$). However, due to the small sample size in the above, no safe conclusions can be exported.

1. Introduction

Smoking causes 80%–90% of all lung cancer deaths (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004), while it increases risk for other cancers (Whiteman & Wilson, 2016) and for cardiovascular disease (Yanbaeva, Dentener, Creutzberg, Wesseling, & Wouters, 2007). Overall mortality among both male and female smokers in the United States is about three times higher than that among similar people who never smoked (U.S. Department of Health and Human Services, 2014). Mechanisms of the adverse effects of smoking have not been completely elucidated yet. Cigarettes contain numerous reactive oxygen and nitrogen species. At the same time, other oxidants are generated endogenously following exposure to tobacco smoking (Bartalis, Chan, & Wooten, 2007). The resulting oxidative stress consists a possible mechanism of the onset and/or progression of smoking-related pathologies (Bjørklund & Chirumbolo, 2017).

Recently, the focus of epidemiologic research has shifted towards understanding the complex interaction of behavioral risk factors as

Abbreviations: FMD, Flow-mediated dilatation; PWV, Pulse wave velocity; ICAM-1, Intercellular adhesion molecule-1; MDA, Malondialdehyde; AOOPs, Advanced oxidation protein products; NO, nitric oxide; CRP, C-reactive protein; LDL, Low density lipoprotein; BMI, Body mass index; BP, Blood pressure

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http://dx.doi.org/10.1016/j.foodres.2017.09.061
Received 9 June 2017; Received in revised form 19 September 2017; Accepted 21 September 2017

determinants of health; this includes the interaction of tobacco consumption and dietary exposures (Shivappa et al., 2015). The assessment of nutritional habits in population studies has demonstrated that smokers and non-smokers differ in the kind of food they eat. Smokers tend to have higher intake of energy, total fat, saturated fat, cholesterol and alcohol (Northrop-Clewes & Thurnham, 2007), lower consumption of fruits and vegetables (Dauchet et al., 2010) and overall lower adherence to the Mediterranean dietary pattern (Hu et al., 2013) compared with non-smokers. This suggests that some of these differences may exacerbate the deleterious effects of components in tobacco smoke on risk of cancer and coronary heart disease.

Since fruits and vegetables contain several nutrient and non-nutrient compounds, including phenolic compounds, vitamins, trace elements and fibers, smokers tend to have lower serum dietary antioxidant concentrations (Yanbaeva et al., 2007) compared with non-smokers. It is well known that fruit and vegetable consumption can protect against reactive oxygen species damage and improve antioxidant status and endothelial function (Gomes-Rochette et al., 2016). Corinthian raisins are small sun-dried fruits, produced almost exclusively in Greece, with significant phenolic content (Kanellos et al., 2013). Recently, the anti-inflammatory and anti-oxidative properties of Corinthian raisins in diabetic patients have been reported, indicating a potential role in cardiovascular disease prevention (Kanellos et al., 2014). Focusing on improving the clinical features and markers of oxidative stress, inflammation and arterial function in healthy smokers, we designed a randomized controlled clinical trial to investigate the effects of diet supplementation with raisins in between meals.

2. Materials and methods

2.1. Study population

Apparently healthy volunteers were invited to participate in the study through a University advertisement and word of mouth. Recruitment was based on the following inclusion criteria: age between 20 and 40 years, normal Body Mass Index (BMI) values (18.5–24.9 kg/m^2), smoking at least 10 cigarettes per day for at least 5 years, low adherence to the Mediterranean type diet indicated by i) MedDietScore values lower than 30 and ii) fruit and vegetable consumption lower than the recommendation of 5 servings daily. As assessed by a medical history questionnaire, physical examination and biochemical and hematological indices, all subjects included in the study were healthy. Exclusion criteria were alcohol abuse or drug use, any medication or vitamin/mineral supplementation or alternative diet (vegetarian, macrobiotic, etc.), pregnancy or lactation prior to the study. An additional exclusion criterion was pre-existence or clinical evidence of any gastrointestinal disease, such as inflammatory bowel disease, gastric ulcer and stomach or intestinal cancer.

The primary end-point was deemed to be malondialdyhyde (MDA), as conventional measure of oxidative damage. The CV in published reports for MDA is 20% (Lai et al., 2005). A sample size of 33 was required to detect a 25% difference between any two treatments for MDA with 80% power assuming a CV of 50% and a two-sided α of 0.05. To allow for a 10% dropout rate over the 4 weeks, a total sample of no $<$ 36 participants was required.

2.2. Study design

All eligible subjects signed an informed consent form after a full review of the inclusion and exclusion criteria and an explanation of the risks and benefits of the study, which were approved by the Ethics Committee of Harokopio University, based on the Helsinki Declaration. In a two-armed, single center, randomized, controlled, 4-week prospective intervention trial, subjects were randomly assigned either to the control arm or to the intervention arm. The allocation of patients in the two arms was random. In this study simple randomization was chosen and the randomization sequence was computer generated. An independent statistician used a computer randomization software. After randomization, the statistician sent the randomization list to the trial principal investigator who completed a participant form for each subject, including the treatment and the subject trial number and put it in a sealed envelope. Blinding of the allocated treatment was maintained to data analysts and was exposed only after the assessment of outcomes. Participants in the intervention arm incorporated in their daily diet the consumption of 90 g of raisins equal to five fruit servings replacing snacks of alike nutritional value (low fat yogurt, mini crackers, or bread with low fat cheese). Raisins were provided in packages of 36 g each amount equal to two fruit servings, kindly donated by the Agricultural Cooperatives Union, Aegion, Greece.

2.3. Anthropometrics and blood pressure (BP)

Anthropometric indices such as body weight (BW), height, waist and hip circumferences (WC, HC) and BMI were recorded both at baseline and after the end of the trial. BW was measured early in the morning in the fasting state with subjects in light clothing without shoes using a flat scale (Tanita WB-110MA, Japan) recorded to the nearest 0.1 kg and height was measured on a stadiometer (Seca Model 220, Germany) recorded to the nearest 0.1 cm. BMI was calculated as weight (in kg) divided by height^2 (in m^2). The WC was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch-resistant tape. HC was measured around the widest portion of the buttocks, with the tape parallel to the floor. All anthropometric measurements were recorded after a $\geq$ 12-hour fast. Diastolic and systolic BP (DBP and SBP in mmHg, respectively) and heart frequency were evaluated at baseline and after the study completion using an electronic sphygmomanometer (OMRON HEM-907 XL, OMRON, Kyoto, Japan). Participants were asked to lie down and relax for a few minutes, after which, two consecutive BP measurements were recorded at an interval of 1–2 min. The recorded value was the mean of the two measurements.

2.4. Dietary history and analysis

Food data were collected. Each participant was asked to keep a 3-day food record (non-consecutive days, including one weekend day). Dietitians trained participants and reviewed unclear descriptions, errors, omissions, or doubtful entries in records and asked the participants to clarify them. The research dietitian supervisor checked all completed records for accuracy. The MedDietScore questionnaire was applied to estimate adherence to the Mediterranean dietary pattern (Panagiotakos, Pitsavos, Arvaniti, & Stefanadis, 2007). Furthermore, during the trial, dietary counseling was monitored by a mid-term non-scheduled phone call receiving a 24-hour dietary recall. To calculate energy intake and macronutrient breakdown (fat, protein, and carbohydrate) nutritional data were analyzed by Nutritionist Pro nutrient analysis software version 5.2.0 (Axxya Systems, Nutritionist Pro, Stafford, TX).

2.5. Clinical analyses

Blood samples were drawn at baseline and at the end of the study (week 4) through a catheter in an antecubital vein after a 12 h overnight fast. Freshly drawn blood samples were used for the determination of glucose, lipid profile, liver enzymes, urea, uric acid, creatinine and total proteins using an automatic analyzer. Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. For assays to determine inflammation and oxidative stress biomarkers, serum and plasma samples were collected, separated by centrifugation at 1800*g* for 10 min at 4 °C, and stored at − 80 °C for subsequent analyses.