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Pilot study

A pilot, randomized controlled trial to examine the health outcomes of raisin consumption in patients with diabetes

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ABSTRACT

Objectives: Dried fruits, like their fresh homologues, contain relatively high concentrations of antioxidants. The aim of this study was to determine the health outcomes of raisin consumption on patients with diabetes.
Methods: We examined the effects of dried grapes (*Vitis vinifera*) cultivated in Greece, namely Corinthian Raisins (CR) on blood pressure, fasting glucose, glucated hemoglobin (HbA_{1c}), lipid peroxidation, high-sensitivity C-reactive protein, antioxidant status, and cytokines in patients with type 2 diabetes mellitus (T2DM). Forty-eight well-controlled patients with T2DM from the diabetes outpatient clinic of our hospital were recruited to a two-armed, randomized, controlled, 24-wk prospective intervention trial in order to examine the health outcomes of CR consumption. All participants were reported to consume less fruits and vegetables than the recommended amount of five servings daily. Participants in the intervention were instructed to consume CR equal to two fruit servings (36 g/d), replacing snacks with similar energy density twice during the day. Anthropometric and blood pressure measurements, assessment of dietary intake, and fasting blood draws were conducted at baseline and at week 24. Also, phenolic compounds present in CR were analyzed in plasma of the patients. *t* Test for parametric data and Mann-Whitney test or Wilcoxon test for non-parametric data were performed. Significance was set at $P < 0.05$.
Results: Body weight, glycemic control, and lipid profile were not affected in either arm. Patients in the CR arm reduced their diastolic blood pressure and increased their total antioxidant potential significantly compared with baseline. The differences between the two groups at week 24 were significant. No change in high-sensitivity C-reactive protein was observed. A significant difference in plasma circulating *p*-hydroxybenzoic acid was observed between groups at the end of the trial.
Conclusions: Our study shows that naturally CR may improve health features in patients with well-controlled T2DM.

Introduction

Fruits containing relatively high concentrations of flavonoids, anthocyanins, and procyanidins, such as grapes, berries, and pomegranate, have shown several health benefits, particularly with respect to antihypertensive effects, inhibition of platelet aggregation, and increasing endothelial-dependent vasodilation. Additionally, flavonoid-rich fruits, such as oranges and grapefruits, are reported to have hypocholesterolemic effects, with little effect on other risk factors being examined [1–3]. Similar to the fresh homologues, dried berries also have been found to decrease atherosclerosis biomarkers in patients with metabolic syndrome [4,5].

Raisins are dried vine fruits (*Vitis vinifera L.*) produced in several geographic regions in the world. Like fresh grapes, they contain fiber but no fat, saturated fat, or cholesterol. They are rich in polyphenols, mainly flavonols, quercetin, and kaempferol, and the phenolic acids, caftaric and coutaric acid [6]. Corinthian Raisins (CR) are small sun-dried berries, colored black to dark blue, produced almost exclusively in Greece, largely exported to England, but also to other European Union

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countries. In one study [7], vanillic acid, as well as caffeic, gallic, syringic, *p*-coumaric, protocatechuic, ferulic acids, and quercetin were detected in CR. A polyphenol extract from CR exhibited cytotoxic activity in AGS (Homo sapiens gastric adenocarcinoma) cell line [8] and in HT29 (Homo sapiens colon colorectal adenocarcinoma) cell line [9]. Also, the in vitro antioxidant and cytoprotective effects in mononuclear cells [10] have been reported. Clinical trials on the health benefits of dried fruits, including dried grapes, in humans are limited. Most recently, the anti-inflammatory and antioxidative properties of dried plums in postmenopausal women were reported, indicating a potential role of dried plums in the prevention of cardiovascular disease (CVD) [11]. A crossover design to evaluate the metabolic response to CR in healthy controls and in patients with diabetes showed that glycemic and insulinemic responses were regulated compared to glucose [12]. The primary aim of the present study was to evaluate the effect of a 24-wk intervention with CR consumption on a daily basis on physical measurements (height, weight, body mass index [BMI], waist circumference [WC], and blood pressure [BP]), blood biomarkers (fasting blood glucose, glycosylated hemoglobin [HbA_{1c}]) and lipid profile in patients with type 2 diabetes mellitus (T2DM). Also, the aim was to assess the effect of the intervention on high-sensitivity C-reactive protein (hs-CRP), cytokines, and the antioxidant status of patients.

Materials and method

Study population

Men and postmenopausal women with T2DM (minimum interval since diagnosis 3 y), selected from the diabetes outpatient clinic of our hospital were recruited to the study. Enrollment began in September 2011, and sample collection from both groups was successfully completed in May 2012. Diagnosis of diabetes was based on the American Diabetes Association criteria [13]. Recruitment was based on the following inclusion criteria: age 40 to 65 y, good glycemic control ($HbA_{1c} < 8\%$), systolic blood pressure (SBP) <180 mm Hg measured on 2 separate days using an OMRON HEM-907XL monitor (OMRON, Kyoto, Japan), diastolic blood pressure (DBP) <110 mm Hg, and overweight to moderately obese with BMI 25 to 35 kg/m^2. Exclusion criteria included more than two fish meals per week or more than 40 g/d of ethanol, the use of fish oil or antioxidant vitamin supplements, a history of alcohol abuse, mental disease, serum triglycerides >307 mg/dL, serum total cholesterol >309 mg/dL, a recent history (within 3 mo) of heart disease, angina, major surgery, myocardial infarction or stroke, liver or renal dysfunction, medical history of abnormal thyroid function, and regular use of nonsteroidal anti-inflammatory or corticosteroid medication drugs. Lipid-lowering drugs were allowed provided the dose was stable 3 mo before screening and the dose did not change during the study. Additionally, the antidiabetic and antihypertensive treatment had to remain stable 3 mo before screening and during the study. Participants volunteered to participate in the study and gave written informed consent. The study received ethics approval from the Harokopio University Research Bioethics Committee (Reference No 30/14.06.2011) in accordance with the Helsinki Declaration.

Decrease in hs-CRP was set as the primary outcome of this study. The sample size was estimated by using data for hs-CRP response to grape seed extract [14]. On the basis of the means and SDs of hs-CRP response to treatment in this previous study, it was estimated that a sample size of 19 participants per group was required to detect a difference of 1.2 mg/L of hs-CRP with a statistical power of 80% at $\alpha = 0.05$. To allow for a 15% dropout rate, we estimated that a sample size of 22 participants in each treatment group would be required. Of the 60 participants enrolled for the trial, 51 were eligible and were randomized to either the control group or the CR-intervention group.

Study design

This study is a two-armed, single-center, randomized, controlled, 24-wk prospective intervention trial. Patients with T2DM were randomly assigned to the control group or the CR group using predefined computer-generated numbers. From the 51 eligible participants, 24 were assigned to the control group and 27 to the CR arm. All participants were reported to consume less fruits and vegetables than the recommended amount of five servings daily. Participants in the CR-intervention arm were instructed to consume CR equal to two fruit servings (36 g/d; serving size equal to 18 g according to Exchange Lists for Diabetes, USDA 2007), replacing snacks with similar energy density twice daily. Participants in the control group were instructed to maintain their usual dietary habits with consumption of vegetables and fruits as reported at baseline. Additionally, the participants in the control group were instructed to totally abstain from consumption of grapes or any raisins. Patients were instructed to complete daily diaries of self-monitored blood glucose. The CR group received the raisins in packs of 36 g, specially prepared for the needs of the trial on a monthly basis. According to data obtained by the certified laboratory of the Agricultural Cooperative Union, Aegion, Greece (HACCP, ELOT-EN-ISO/IEC 17025:2005), nutritional composition of 36 g CR was as follows: energy 115.2 kcal; carbohydrates 26.3 to 27.7 g; sugars 23.8 to 25.2 g; protein 0.9 g; fat traces; and dietary fibers 2.4 g. Also, according to one study [7], total polyphenol content in 36 g was 54.4 to 88.6 mg, equivalent to gallic acid. Participants met monthly for instructions regarding CR intake, compliance monitoring, and for additional CR supply. Laboratory staff, statistical analysts, and study investigators were blinded to group assignment throughout data collection and analysis.

Anthropometrics and blood pressure

Anthropometric measurements such as body weight, height, WC and hip circumference, and SBP and DBP were recorded by trained personnel in the Laiko University Hospital at baseline and at the end of the trial. Body weight was measured early in the morning in the fasting state with patients wearing light clothing without shoes and using a flat scale (Tanita WB-110MA, Japan), and was recorded to the nearest 0.1 kg; height was measured in a stadiometer (Seca Mode 220, Germany), recorded to the nearest 0.1 cm. BMI was calculated as weight (in kg) divided by height squared (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. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. SBP and DBP were measured in millimeters of mercury (mm Hg) using an OMRON HEM-907XL device. Participants were asked to lie down and relax for a few minutes, after which, three consecutive BP measurements were recorded at an interval of 1 to 2 min. Pulse rate was measured automatically using an electronic pulse meter (OMRON HEM-907XL, OMRON, Kyoto, Japan). Fasting blood draws were conducted by a certified phlebotomist on the screening visit (week 0) and at 24 wk of the study.

Dietary analyses

A weekly food-frequency questionnaire was applied at the screening visit to ensure that enrolled participants had similar dietary habits. Well-trained dietitians with education in foods available in the marketplace and in preparation practices, including prevalent regional or ethnic foods, conducted 24-h recalls by in-person interviews using a paper-and-pencil form at baseline and post-intervention. During the trial, for purposes of compliance monitoring, dietitians conducted unannounced phone interviews. Diet records were analyzed using Diet Analysis Plus 4.0, which is based on the Food Composition Database ESHA Research, 1999.

Clinical analyses

Blood samples were drawn at baseline and at the end of the study (week 24) through a catheter in an antecubital vein after a 12-h overnight fast. Freshly drawn blood samples were used for determination of glucose, lipids, liver enzymes, and blood urea nitrogen using an automatic analyzer. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [15]. HbA_{1c} was measured using high-performance liquid chromatography. 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. Albumin and uric acid were measured photometrically according to the instructions of the manufacturing company.

Inflammation biomarkers

High-sensitivity quantikine human enzyme-linked immunosorbent assay (ELISA) kits were used to measure tumor necrosis factor (TNF)-α and interleukin (IL)-6 (R&D Systems, Abingdon, UK) with sensitivity ranges from 0.550 to 2.816 pg/mL and 0.016 to 0.110 pg/mL, respectively. hs-C-reactive protein ELISA kit was obtained by IBL (Hamburg, Germany) with detectable limits of 0.02 μg/mL. Quantikine human ELISA kits were used to measure leptin and adiponectin (R&D Systems, Abingdon, UK) with sensitivity ranges from 2.2 to 77.3 ng/mL and 0.079 to 0.891 ng/mL, respectively. The intra-assay coefficients of variability were <7 % in all markers.