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Short communications

## Almond ingestion contributes to improved cardiovascular health in sedentary older adults participating in a walking intervention: A pilot study

### C.S. Johnston<sup>a,\*</sup>, K.L. Sweazea<sup>a,b</sup>, E. Schwab<sup>a</sup>, E.A. McElaney<sup>a</sup>

<sup>a</sup> School of Nutrition and Health Promotion, Arizona State University, Phoenix, AZ 85004, United States
<sup>b</sup> School of Life Sciences, Arizona State University, Tempe, AZ 85287, United States

#### A R T I C L E I N F O

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#### ABSTRACT

A small pilot study was conducted to assess synergy between almond ingestion and a walking intervention on cardiovascular health in sedentary adults. Participants (n = 12) followed an 8-week walking protocol (target: 10,000 steps/day), and for study weeks 6–8, participants were randomly assigned to almonds (ALM, 70 g/d) or an isocaloric control butter spread (CON). Protocol adherence was verified by plasma vitamin E measurement. Participant step count increased 23% from baseline levels for weeks 3–5 (p = .015) and remained  $\geq$ 9800 steps/day for the study duration. Although improvements in cardiovascular health were not demonstrated by the walking protocol alone, total and LDL cholesterol were reduced in the ALM group only during study weeks 6–8 (-6 to -10%; p < .05), and lipid peroxidation tended to fall in the ALM group only as well (-25%; p = .082). These data highlight the value of including almonds in the diet when recommending a walking protocol for improving health.

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#### 1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in the U.S. and worldwide. Although high blood pressure is the leading risk factor for CVD (attributable fraction, 40.6%), a poor diet and insufficient physical activity rival smoking as risk factors for CVD (attributable fractions, 13.2%, 11.9%, and 13.7% respectively) (Go et al., 2014). Hence, lifestyle modifications that include physical activity and a cardio-protective diet represent the foundation for CVD prevention and treatment for all individuals even those on drug therapy (Stone et al., 2014). Substantial evidence from epidemiological studies have established an inverse relationship between physical activity and CVD risk with even simple walking showing a negative dose-dependent association with CVD and all-cause mortality (Qiu et al., 2014; Smith, Wingard, Smith, Kritz-Silverstein, & Barrett-Connor, 2007; Zheng et al., 2009). The protective effect of aerobic activity for reducing CVD risk is attributed in part to improved vascular endothelial function (Pierce, Eskurza, Walker, & Fay, 2011; Sanders et al., 2015).

Of various cardio-protective dietary factors, regular nut consumption is particularly aligned with reduced CVD risk, likely a reflection of the many bioactive constituents of nuts including unsaturated fatty acids, vegetable proteins, minerals, vitamins, antioxidants, and phytochemicals (Mozaffarian, Appel, & Van Horn, 2011). Large epidemiological studies have consistently demonstrated an inverse association between nut ingestion and CVD rates and CVD mortality across age, gender, locality, and profession (Mayhew, de Souza, Meyre, Anand, & Mente, 2016; 10. Brown et al., 2015; Ros, 2015). Furthermore, randomised, controlled trials showed that nuts have beneficial effects on numerous mediators of CVD including blood pressure, blood lipids, inflammation, oxidative stress, and endothelial function (Colpo et al., 2014; Katz et al., 2012; Orem et al., 2013; Sabaté, Oda, & Ros, 2010). Many investigations have focused specifically on almonds due to their particularly rich bioactive nutrient content, including high levels of fiber, vitamin E, arginine, and sphingolipids (Berryman, Preston, Karmally, Deckelbaum, & Kris-Etherton, 2011). In one trial, the addition of almonds to the National Cholesterol Education Program's Step 1 diet reduced total cholesterol and low-density lipoprotein cholesterol to a greater degree than the Step 1 diet alone (Sabaté, Haddad, Tanzman, Jambazian, & Rajaram, 2003). Almonds incorporated into a cholesterol-lowering control diet lowered the inflammatory markers CRP and E-selectin as compared to the same diet without almonds (Rajaram, Connell, & Sabaté, 2010). As for aerobic activity, the cardio-protective effect







<sup>\*</sup> Corresponding author at: School of Nutrition and Health Promotion, Arizona State University, 500 N. 3rd Street, Phoenix, AZ 85004, United States.

*E-mail addresses*: carol.johnston@asu.edu (C.S. Johnston), karen.sweazea@asu.edu (K.L. Sweazea), emschwab@asu.edu (E. Schwab), emcelane@asu.edu (E.A. McElaney).

of almonds and nuts in general has been linked in part to improvements in endothelial function (Choudhury, Clark, & Griffiths, 2014; Jamshed & Gilani, 2014; Rajaram et al., 2010).

Although both walking and almond consumption are associated with improved cardiovascular health, there are no studies that have examined possible synergy between these strategies for improving cardiometabolic biomarkers. The objective of this 8week pilot study in healthy, sedentary adults was to determine if therapeutic synergy existed between daily almond ingestion (70 g) and increased step counts (target: 10,000 steps per day) as indicated by key markers of cardiovascular health (blood lipids, blood pressure, C-reactive protein [CRP], oxidative stress, flow-mediated dilation [FMD], and total nitrates and nitrites [NOx] bioavailability). Participants increased physical activity for the first five weeks of the study, and for the final three weeks of the study, participants combined increased physical activity with the ingestion of almonds or a control treatment.

#### 2. Materials and methods

#### 2.1. Participants

Male participants between 45 and 60 years of age and postmenopausal women up to 60 years of age who reported sitting >8 h daily, a characteristic associated with risk for CVD mortality independent of physical activity levels (Katzmarzyk, Church, Craig, & Bouchard, 2009), were recruited for this pilot trial from the Phoenix metropolitan area via listservs, emails, and flyers. Volunteers were eligible if they answered 'no' to all questions on the Physical Activity Readiness Questionnaire and did not report active chronic disease (including cancer, heart disease, asthma, arthritis, and hypertension). Individuals who reported nut, gluten, or other food allergies; cigarette use within the past year; specific medication use (nitrate vasoactive hypertensive medications, nitroglycerin, beta-blockers, and calcium channel blockers); or who were unwilling to participate in an 8-week walking intervention or comply with testing protocols were excluded from participation. Qualifying individuals (n = 15) were invited to the test site to verify eligibility, and all participants provided written informed consent. This study was approved by the Arizona State University Institutional Review Board.

#### 2.2. Study design

The 8-week intervention was composed of the walking phase (weeks 1-5) and the walking + almonds phase (weeks 6-8). Participants reported to the test site one week prior to the start of the intervention and were fitted with a pedometer which they were instructed to wear until the study was complete. At this initial visit, participants were instructed to complete two 3-day diet records, one record prior to the start of the intervention and one record during the final week of the study. With exception to the study foods, participants were instructed to restrict all nut consumption (including nut milks) to  $\leq 2$  servings per week until the study was complete. Participants returned to the test site after one week (study baseline) to return their initial diet record and to download their pre-study pedometer data. Participants were instructed to fast for this visit (no food or beverage with the exception of water for 10 h). At this visit a blood sample was collected, and FMD, blood pressure, and anthropometric measurements were taken. All participants received instructions to increase step count to 10,000 steps daily over the next two weeks, the first two weeks of the intervention, and to maintain this step count for the duration of the study. At week 5, participants were stratified by gender, age, body weight, and baseline step count and randomly assigned (by coin toss) to one of two diet intervention groups: almonds (ALM; 2.5 oz. raw almonds daily providing 400 kcal, 35 g fat, 15 g carbohydrate, 7.5 g fiber, and 18 mg vitamin E) or cookie butter (CON; 4 tbsp. cookie butter daily providing 360 kcal, 24 g fat, 32 g carbohydrate, 0 g fiber, and <1 g vitamin E). The raw whole almonds were graciously provided by the Almond Board of California in 2.5 oz. [70 g] serving packets, and the cookie butter (Speculoos Cookie Butter, Trader Joe's, Monrovia, CA) was donated by Trader Joe's groceries. The almonds or cookie butter allotments were ingested daily during study weeks 6-8. The cookie butter was presented to participants as 'nut butter' and the label was removed. Participants returned to the test site at weeks 5 and 8 in a fasted state for additional blood sampling, pedometer downloads, and study measurements. The second 3-day diet record was collected at week 8. Aside from the study foods and walking protocol, participants were instructed to maintain their normal dietary patterns and physical activity habits.

#### 2.3. Laboratory analyses

FMD and blood pressure measures were conducted at baseline and trial weeks 5 and 8 by a certified sonographer. FMD was performed using high resolution 2 K and Doppler ultrasound (HDI 5000, ATL Philips Ultrasound). The cuff, appropriate to the size for the limb, was distally positioned to the ultrasound probe at the brachial artery. The cuff was then inflated to ~50 mm Hg above systolic arterial pressure for 5 min to generate a reactive hyperemic stimulus which is regarded as primarily endothelium mediated and NO dependent (Harris, Nishiyama, Wray, & Richardson, 2010). To ensure accuracy of measurement, probe location was recorded for each participant with repeated measures at the same site.

At the same time points that FMD was measured, venous blood was sampled and anthropometric measurements were performed. Plasma samples for NOx analyses were pre-filtered using 30 molecular weight cut-off microcentrifuge filters according to the manufacturer's protocol (Millipore, Billerica, MA) following the Griess method (Cat. No. 780001, Cayman Chemical, Ann Arbor, MI). Thiobarbituric acid reactive substances (a measure of lipid peroxidation) were analysed using a commercial colorimetric assay kit (Cat. No. 0801192, ZeptoMetrix Corporation, Buffalo, NY) following the manufacturer's instructions. Plasma vitamin E was assessed to indicate protocol adherence; analyses were performed by standard HPLC methodology as described previously (Smith et al., 2011). Height was measured using a stadiometer. Body weight and body mass index (BMI) were obtained using a Tanita scale (TBF-300A Body Composition Analyser).

#### 2.4. Statistical analyses

Data are presented as the mean ± SD. Significance was set at p < .05, and all analyses were performed using SPSS v.22 Statistical Analytical System (SAS, Chicago, IL). Mann-Whitney independent group tests were employed to compare variables between groups at baseline, and Wilcoxon signed ranks tests were used to compare change during the first 5 weeks of the study (the walking phase). Analysis of Covariance (ANCOVA), controlling for baseline and change in steps, was used to measure change in cardiometabolic biomarkers between groups at week 8 versus week 5 (the walk ing + almonds phase) and to determine the effect size (ES: partial eta-squared  $\eta_p^2$ ). The Shapiro-Wilk test was utilised to assess normality, and the data were transformed as necessary.

#### 3. Results

Fifteen adults were enrolled in the study at the initial screening visit; however, one participant was excluded at this visit due to

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