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Effects of short-term walnut consumption on human microvascular function and its relationship to plasma epoxide content☆

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Abstract

Improved vascular function after the incorporation of walnuts into controlled or high-fat diets has been reported; however, the mechanism(s) underlying this effect of walnuts is(are) poorly defined. The objective of the current study was to evaluate the acute and short-term effects of walnut intake on changes in microvascular function and the relationship of these effects to plasma epoxides, the cytochrome-P450-derived metabolites of fatty acids. Thirty-eight hypercholesterolemic postmenopausal women were randomized to 4 weeks of 5 g or 40 g of daily walnut intake. All outcomes were measured after an overnight fast and 4 h after walnut intake. Microvascular function, assessed as the reactive hyperemia index (RHI), was the primary outcome measure, with serum lipids and plasma epoxides as secondary measures. Compared to 5 g of daily walnut intake, consuming 40 g/d of walnuts for 4 weeks increased the RHI and Framingham RHI. Total cholesterol and low- and high-density cholesterol did not significantly change after walnut intake. The change in RHI after 4 weeks of walnut intake was associated with the change in the sum of plasma epoxides (r=0.65, P=.002) but not with the change in the sum of plasma hydroxyeicosatetraenoic acids. Of the individual plasma epoxides, arachidonic-acid-derived 14(15)-epoxyeicosatrienoic acid was most strongly associated with the change in microvascular function (r=0.72, P<.001). These data support the concept that the intake of walnut-derived fatty acids can favorably affect plasma epoxide production, resulting in improved microvascular function.

Keywords: Walnuts; Vascular function; Oxylipin; alpha-Linolenic acid; Linoleic acid

1. Introduction

Epidemiological evidence suggests that the intake of tree nuts can provide significant cardiovascular benefits [1,2]. Numerous investigators have reported that acute and short-term intake of walnuts can be associated with reductions in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, reductions in blood pressure and improved vascular function [3–8]. However, the mechanism(s) underlying the above physiological changes has(have) not been well characterized. Measures of vascular function, such as flow-mediated dilation (FMD) and peripheral arterial tonometry (PAT), are used as noninvasive surrogate outcome measures, the responses to which can be associated with a number of cardiovascular risk factors [9]. Improvements in vascular function have been reported in several dietary interventional trials after walnut intake [1,4,6,7,10]. Notably, a variety of study designs have been used that include the assessment of vascular function after an overnight fast [7,10] or during the postprandial period with the walnuts incorporated or consumed with a high-fat meal [3,4,11]. The latter studies have explored the potential of walnuts to reduce lipidemia-induced vascular dysfunction, an effect of walnuts that may represent a distinct mechanism of action that is separate from the vascular effects induced during the fasted state [12].

Walnuts can be a substantial source of the essential fatty acids omega-3 alpha-linolenic acid (ALA) and omega-6 linoleic acid (LA) [1], as well as several other nutrients that have been postulated as being valuable for reducing the risk for cardiovascular disease (CVD) [1].

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Only a small number of studies have examined the relationships between specific components within walnuts and vascular function in human subjects [5,6]. An enhanced FMD response, which is a measure of macrovascular function, has been reported when walnuts, walnut oil and/or ALA-rich flaxseed oil were added to the diet for 6 weeks [5]. An acute (single-feeding) noted a greater microvascular response, as measured by PAT, after intake of walnut oil compared to walnut skin consumption [6]. Both studies suggest that components within the oil fraction of walnuts can influence the vascular response [5,6].

The endothelium plays a critical role in maintaining vascular homeostasis and produces a number of mediators involved in the regulation of vascular tone. These include vasodilators such as nitric oxide (NO), prostacyclin and endothelial-derived hyperpolarizing factor (EDHF), and vasoconstrictors such as endothelin-1. These mediators can have profound influences on platelet reactivity, inflammatory responses and vascular smooth muscle cell remodeling. Cardiovascular risk factors, particularly lifestyle choices such as diet, are thought to modulate the balance of these mediators. The mechanisms by which walnut intake influences vascular function have not been defined, but they may include lipoprotein-induced modulation of vascular hemostasis, particularly with regards to a large group of bioactive lipids collectively termed oxylipins [13]. Known bioactive oxylipins are the cyclooxygenase (COX)-, lipoxygenase (LOX)- and cytochrome P450 (CYP)-derived metabolites of arachidonic acid (AA), and include thromboxane and prostacyclin, known modulators of platelet and vascular reactivity, and epoxyeicosatrienoic acids (EpETrEs or EETs), which have been proposed as putative EDHFs that regulate microvascular tone [13,14]. In addition to EpETrE formation by CYP epoxygenase, CYP ω/ω-1 hydrolases produce hydroxyeicosatetraenoic acids (HETEs) that are considered vasoconstrictive and inflammatory [15]. Furthermore, soluble epoxide hydrolase (sEH) metabolizes EpETrEs to form dihydroxyeicosatrienoic acids, which are generally thought to be less vasoactive. Dysregulation of CYP epoxygenases and hydrolases has been observed in diabetes [16], in hypertension [17,18] and in patients with stable CVD [19,20].

While epoxides derived from AA are the most extensively studied and are commonly inferred to be the principal bioactive agents, most studies have not been designed to address the potential bioactivity of other fatty-acid-derived epoxides. Considerable interest has been given to the potential discovery of sEH inhibitors that may potentiate the beneficial vascular effects of epoxides [21]. Recent studies have suggested that ALA-rich flaxseed may be of benefit to those with hypertension, as a decrease in sEH metabolites was associated with improved blood pressure after 6 months of flaxseed intake [18]. Given the above, the primary aim of the current study was to evaluate the acute and short-term effects of walnut intake on microvascular function in a population at increased cardiovascular risk; secondly, we sought to assess the effects of walnut intake on lipid profiles including an examination of relationships between microvascular function and plasma epoxides.

2. Methods

2.1. Participants

Thirty-eight postmenopausal women (50–70 years of age) were recruited from the greater Sacramento area via public advertisements. After providing written informed consent, potential participants were screened by a multiple-phase process that included a telephone interview, a lipid and metabolic panel, and PAT. Inclusion criteria were a body mass index (BMI) <35 kg/m^2, postmenopausal state (defined as the absence of a menstrual cycle for at least 1 year and a follicle-stimulating hormone level between 23 and 116.3 IU/L) and a fasting TC level ≥5.18 mmol/L. Exclusion criteria included allergies to walnuts or other nuts, use of prescription medications except for treating hypothyroidism, use of dietary supplements except standard multivitamin/mineral formulas, cigarette smoking, and a history of CVD or other chronic diseases. This dietary intervention trial was registered (NCT01235390) at ClinicalTrials.gov, with the study protocol approved by the Institutional Review Board of the University of California, Davis.

2.2. Study design

Participants were randomized into a 4-week, parallel-arm dietary intervention trial by block design to consume 5 g or 40 g of walnuts daily for 4 weeks. Randomization was performed by the study coordinator following a predetermined plan formulated via a Web-based random number generator (www.randomization.com). One week prior to and during the intervention period, participants were instructed to refrain from consuming any type of nuts, except for the walnuts provided, as well as to refrain from the use of aspirin and other over-the-counter pain relievers. To assess the short-term (4 weeks) effects of walnut intake, assessments were made prior to the initiation of daily walnut intake and at the end of the 4-week intake period. For each assessment, participants arrived at the facility in the morning after an overnight fast. After a 30-min rest period, a PAT measurement was performed, followed by blood collection from the antecubital vein. After this initial set of baseline measurements, the acute (single intake) response of walnut consumption was also determined, with the subjects consuming their assigned amount of walnuts, followed by an additional PAT measurement and blood draw 4 h later.

Each participant received walnuts prepackaged into 5- or 40-g daily servings and were instructed to consume one serving daily for 4 weeks. The 40-g level of daily walnut intake was chosen based on the level of daily intake suggested in the 2004 Food and Drug Administration qualified health claim for walnuts and cardiovascular health [22], and also reflected the level and intake period (4 weeks) previously observed to improve postprandial vascular dysfunction induced by a high-fat meal [3]. The goal of the current study was to determine the vascular effects of walnuts when incorporated into the participant's habitual diet as a whole food. As this study design makes blinding difficult without significantly changing the form of the walnut (i.e., making it into a powder, baking, etc.), we chose to utilize a dose–response design, asking the subjects to consume either 40 or 5 g/d of walnuts as either a snack or incorporating the walnuts, without cooking them, into their meal.

Compliance was monitored by a self-administered log. Food intake was assessed from the compliance log as well as two 3-day food records that included two weekdays and one weekend day, consecutively, that were completed before each study day visit. Food records were analyzed using The Food Processor SQL (version 10.1.0; ESHA Research, Salem, OR, USA), which provides nutrient information for 26,000 food items compiled from the USDA nutrient database for standard reference, manufacturer and restaurant nutrition information, and other source information. Compared to values obtained from laboratory analysis, previous versions of this software have a reported accuracy within 5% of the measured macronutrient values to include the fatty acid classes [23]. In addition, nutrient estimates have strongly correlated to nutrient data from the First National Health and Nutrition Examination Survey [24].

2.3. Assessment of microvascular function and augmentation index

Microvascular function, assessed as reactive hyperemia index (RHI), was measured using PAT (EndoPAT 2000; Itamar Medical, Caesaria, Israel). Before each RHI measurement, participants relaxed in a supine position for at least 30 min in a quiet, temperature-controlled room, with speaking and movement kept to a minimum. A plethysmographic-like biosensor was placed on the middle finger of