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The effect of dietary walnuts compared to fatty fish on eicosanoids, cytokines, soluble endothelial adhesion molecules and lymphocyte subsets: a randomized, controlled crossover trial ☆

Yu-Lan Chiang [a], Ella Haddad [a,*], Sujatha Rajaram [b], David Shavlik [b], Joan Sabaté [a]

[a] Department of Nutrition, Loma Linda University, Nichol Hall 1102, Loma Linda, CA 92354, USA
[b] Department of Epidemiology, Loma Linda University, Nichol Hall 2005, Loma Linda, CA, 92354, USA

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ABSTRACT

We tested the hypothesis that walnut consumption can exert effects on markers of inflammation and endothelial activation similar to those produced by fish consumption. In a crossover dietary intervention trial, 25 normal to mildly hyperlipidemic men and women were randomly assigned to one of three isoenergetic diets: a walnut diet incorporating 42.5 g of walnuts per 10.1 mJ 6 times per week (1.8% of energy n-3 fat); a fish diet providing 113 g of fatty fish per 10.1 mJ 2 times per week (0.8% of energy n-3 fat), or a control diet (no nuts or fish, 0.4% of energy $n-3$ fat) for 4 weeks on each diet. Both the walnut and fish diets inhibited circulating concentrations of prostaglandin E metabolite (PGEM) and 11-dehydro thromboxane B2, but demonstrated no effect on blood interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) or the number of circulating lymphocyte subsets. On the walnut diet the proportion of plasma phospholipid α-linolenic acid (ALA) increased 140% and arachidonic acid (AA) decreased 7% compared to both the control and fish diets. The proportion of plasma phospholipid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased about 200% and 900% respectively on the fish diet relative to either the control or walnut diet. The walnut diet inhibited E-selectin by 12.7% relative to the fish diet, and the fish diet inhibited secretory intercellular adhesion molecule-1 (s-ICAM-1) by 4.5% relative to the control diet. Both walnuts and fish in commonly consumed amounts may have modest albeit distinct effects on circulating adhesion molecules.

1. Introduction

Epidemiological and clinical evidence indicates that fish consumption is protective against cardiovascular disease (CVD) mortality and morbidity [1,2]. Inflammation is pathophysiological for CVD and the cardioprotective benefits of fish are attributed to the anti-inflammatory effect of the n-3 polyunsaturated eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6, n-3) found in marine foods and oils. Walnuts, on the other hand, are one of the few commonly consumed plant foods that contain substantial quantities of the n-3 fatty acid α-linolenic acid (ALA, 18:3n-3). Clinical trials consistently demonstrated that the incorporation of walnuts in healthy diets lowered blood lipids and other risk factors related to CVD morbidity [3–7].

Dietary long chain polyunsaturated fatty acids are substrates of eicosanoids, cytokines, and other lipid mediators, and as such play an important role in the regulation of inflammation and related processes [8]. Membrane phospholipids of inflammatory cells contain a high proportion of arachidonic acid (AA, 20:4n-6), a major precursor of the inflammatory prostaglandins and thromboxanes. Increased dietary consumption of n-3 fatty acids leads to their increased incorporation in cell phospholipids at the expense of AA, resulting in decreased substrate for synthesis of pro-inflammatory mediators. Also, several eicosanoids formed from n-3 fats are less potent inflammatory agents than those formed from AA. Although inflammation is a normal physiological process that is self-regulated, self-limiting and essential to health, loss of control may lead to impaired function and disease. Dietary intakes of most individuals living in Western countries tend to be low in n-3 polyunsaturated fats, a condition which may impair regulation and promote pro-inflammatory and pro-aggregatory states [9]. Cross-sectional studies showed that higher intakes of EPA and DHA were independently related to lower concentrations of C-reactive protein (CRP) in populations with a diet rich in

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* Corresponding author. Tel.: +909 558 4598; fax: +909 558 4095.
E-mail address: ehaddad@llu.edu (E. Haddad).

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marine products [10,11], and fish consumption was inversely related to circulating concentrations of CRP and interleukin-6 (IL-6) in a multiethnic population [12].

Walnuts are relatively rich in both linoleic acid (LA, 18:2n-6) and ALA, and exhibit a favorable n-6:n-3 ratio of 4:1 [13]. Walnut-supplemented diets decreased plasma secretory vascular adhesion molecule-1 (VCAM-1) but not CRP [14], and decreased postprandial soluble E-selectin concentrations in patients with hypercholesterolemia [15]. There have been no reports on the effect of walnut-enriched diets on inflammation and adhesion biomarkers in normal to mildly hyperlipidemic individuals typical of the general public.

The American Heart Association (AHA) guidelines currently include a recommendation to increase fatty fish consumption to 2 or 3 servings per week [16]. However, fish is not part of the daily US diet and the health benefits of n-3 PUFAs in fish may be somewhat offset by contamination of fish with methyl mercury and polychlorinated biphenyls. Additionally, populations who avoid animal foods do not receive a direct source of EPA and DHA in their diet. Although conversion is limited, for many individuals endogenous biosynthesis from ALA is the major contributor to the body's supply of EPA and DHA [17].

Whereas the anti-inflammatory effect of EPA and DHA from seafood and fish oil is generally accepted, the effect of ALA from plant sources such as walnuts is not established. The aim of the present study was to evaluate the effect of incorporating walnuts and fatty fish containing plant compared with marine n-3 fatty acids into healthful diets on circulating lymphocyte cell numbers, plasma phospholipid fatty acid profile, and concentrations of selected inflammatory eicosanoids, cytokines and soluble adhesion molecules.

2. Materials and methods

2.1. Subjects and study design

This study was a sub-study of one which tested the effects of walnuts compared to fish on blood lipids and lipoproteins and study details were previously reported [6]. The study was a randomized, controlled, single-blind, crossover trial in which 25 adult volunteers, age 23–65 years, completed a feeding study conducted at the UD Register Research Kitchen in Loma Linda, CA. Participants consumed 3 diets for 4 weeks each for a total controlled feeding period of 12 weeks. The test diets were: (1) a control diet which excluded nuts and seafood; (2) a fish diet which contained two servings of fatty fish two times per week; and (3) a walnut diet which incorporated walnuts 6 days per week.

Participants were screened by a multi-phase process that included a telephone interview, an informational group meeting, one-on-one interviews and a preliminary fasting blood lipid test. The study protocol was approved by the Institutional Review Board of Loma Linda University, Loma Linda, CA, and informed consent was obtained from all participants. To be eligible for the study, participants had to be normal to moderately lipidemic (serum cholesterol <300 mg/dL, serum triglyceride <300 mg/dL) and nonsmoking adults who reported no weight change for the previous 6 months. Individuals were excluded if they used vitamin E, fish oil, or flaxseed supplements; consumed nuts ≥ 2 times per week; drank alcoholic beverages ≥ 2 times per week; drank caffeinated beverages ≥ 3 times per day; were diagnosed with an endocrine or metabolic disease; or, used non-steroidal anti-inflammatory medications. The participants were instructed to maintain their habitual level of physical activity throughout the study.

2.2. Dietary intervention

All treatment diets adhered to current dietary guidelines and provided similar amounts of macronutrients and saturated fat (8–9% of energy). The fish diet offered two 113 g (4 oz) servings of fatty fish per 10.1 mJ (2400 kcal) 2 times per week, whereas the walnut diet incorporated 42.5 g (1.5 oz) of walnuts per 10.1 mJ (2400 kcal) of diet 6 days per week, and, the control diet did not contain any walnuts or fish. Current recommendations such as those of the American Heart Association provided the rationale for the amount of fish and those of FDA's qualified health claim for walnuts justification for the walnut dose [6]. The study featured a controlled feeding protocol with all meals prepared, weighed and served at the university's metabolic kitchen. Sunday through Friday of each week, participants ate breakfast and dinner at the metabolic kitchen, whereas lunch, snacks and Saturday meals were packaged for each subject. Daily menus were formulated at different energy levels, ranging from 1800 kcal/d to 3600 kcal/d, according to the various caloric needs of the subjects.

During all three diet periods, subjects were given diaries to record deviations from protocol such as illness, medications use, or extra foods and drinks consumed. Dietary compliance was assessed by direct observation during meal times and by weekly examination of subjects' diaries. Body weight was recorded everyday during the run-in period and twice a week thereafter. Daily energy intakes were adjusted when necessary to maintain body weight.

2.3. Blood sample collection

Fasting venous blood samples were taken at the end of each of the 3 4-week diet periods. Heparinized whole blood was used immediately for lymphocyte sub-set analysis. Other blood samples were centrifuged and aliquots of serum and plasma were aliquoted and maintained at $-80\,^{\circ}C$ until the analysis. We used fasting blood for analysis of prostaglandin E metabolite (PGEM), 11-dehydro thromboxane B2 (TXB2), IL-1β, IL-6, CRP, TNF-α, secretory intercellular adhesion molecule-1 (sICAM-1), and sE–selectin concentrations. All samples were examined in duplicate.

2.4. Plasma fatty acid composition

Analysis of plasma phospholipid fatty acid composition was carried out by Lipomics Technologies Inc. (Sacramento, CA) according to published procedures [18]. Lipids were extracted from plasma using chloroform/methanol (2:1 vol) and individual lipid classes were separated by preparative thin-layer chromatography. The lipid fractions were then transesterified with methanolic HCl in sealed vials under nitrogen. Fatty acid methyl esters were separated and quantified by capillary gas chromatography (GC) Hewlett-Packard instrument model 6890 (Wilmington, DE) equipped with a 30 m capillary column DB-225MS (J&W Scientific, Folsom, CA) and an Agilent Technologies (Palo Alto, CA) flame ionization detector.

2.5. Lymphocyte subsets

Lymphocyte numbers and subset determinations were performed at the Histocompatibility-Clinical Flow Cytometry Laboratory of Loma Linda University Medical Center (Loma Linda, CA). Due to testing capacity limitations, lymphocyte subset determinations were obtained from 9 male participants of the study. Sample preparation for whole blood was by the lyse-wash method using the following monoclonal antibodies: anti-CD3 conjugated to fluorescein isothiocyanate and anti-CD19, anti-CD4