Daily Consumption of Oregon Hazelnuts Affects α-Tocopherol Status in Healthy Older Adults: A Pre-Post Intervention Study

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ABSTRACT

Background: Inadequate vitamin E and magnesium intakes are of concern for older adults owing to the associated incidence of age-related diseases.

Objective: This study was designed to determine the extent to which a 16-wk intervention with hazelnuts alters vitamin E and magnesium status in a group of older men and women, and used a pre-post intervention design without a control group to adjust for temporal changes.

Methods: Participants (n = 32 including 22 women; mean ± SD age: 63 ± 6 y) consumed hazelnuts (∼57 g/d) for 16 wk. Blood and urine samples and anthropomorphic measures were taken at the start and end of the intervention to determine plasma concentrations of α-tocopherol and serum concentrations of magnesium, lipids, glucose, insulin, and high-sensitivity C-reactive protein along with urinary vitamin E metabolites; several other micronutrients were measured by a lymphocyte proliferation assay. There were 3 primary endpoints, calculated as the mean changes in measurements between baseline and the end of the 16-wk intervention for 1) plasma α-tocopherol, 2) urinary α-carboxyethyl hydroxychromanol (α-CEHC; an α-tocopherol metabolite), and 3) serum magnesium.

Results: Hazelnut consumption increased concentrations of the urinary α-tocopherol metabolite α-CEHC (mean ± SD: 0.84 ± 0.45 to 1.14 ± 0.50 µmol/g creatinine; P = 0.0006). In addition, hazelnut consumption increased serum concentrations of magnesium (+2.1%, P = 0.05), decreased concentrations of fasting glucose (−3.4%, P = 0.03) and LDL cholesterol (−6.0%, P = 0.02), and decreased total:HDL cholesterol ratios (−4.5%, P = 0.009). No significant changes were observed in blood pressure, lymphocyte proliferation assays, and serum concentrations of insulin, high-sensitivity C-reactive protein, triglyceride, α-tocopherol, or HDL cholesterol.

Conclusions: Consuming hazelnuts improves a biomarker of vitamin E status in older adults. Vitamin E is a shortfall micronutrient, as identified by the Dietary Guidelines for Americans 2015–2020, which frequently is consumed at levels less than the Estimated Average Requirement of 12 mg/d; thus, hazelnuts should be considered as part of a healthy dietary pattern. This trial was registered at clinicaltrials.gov as NCT03485989.

Keywords: vitamin E, tree nuts, heart-healthy diet, carboxyethyl hydroxychromanol, magnesium

Introduction

In the United States, many older adults do not consume adequate amounts of vitamin E and magnesium from their habitual diet. National surveys have revealed that >90% of adults do not reach the Estimated Average Requirement (EAR) for vitamin E [12 mg/d (1)] without the use of dietary supplements or fortified foods (2), and this is especially true for adults >50 y of age (3, 4). Similarly, dietary intakes of magnesium by older adults [the EARs for adults 51–70 y old are 350 mg/d for men and 265 mg/d for women (5)] are also inadequate, because recent estimates show >60% of individuals aged >50 y do not consume EAR recommended intake levels of this essential mineral (4). For both vitamin E and magnesium, along with several other vitamins and minerals, these nutritional deficits increase in frequency and severity after the age of 70 y (3, 4). Because lower vitamin E and magnesium concentrations are associated with increased risks of frailty and incidence of age-related diseases, including Alzheimer disease (6–9), strategies for improving suboptimal status in both of these micronutrients are important determinants of health.

Many nuts, including hazelnuts, are sources of vitamin E and magnesium. Nuts also contain numerous other vitamins and minerals, fiber, unsaturated fats, and a variety of phytochemicals (10). Hazelnuts, in addition to being excellent sources of α-tocopherol and magnesium, are particularly high in copper and manganese (>25% of the DRI), contain 2.7 g of dietary...
furfural, and have the highest content of folate and proanthocyanidins of any nut (11). As demonstrated in various clinical trials, the health benefits of consuming hazelnuts include lowering blood glucose concentrations (12), beneficially altering blood lipids (13–17), and decreasing oxidative stress biomarkers (11, 18). High nut consumption also reduces several risk factors for developing cardiometabolic diseases (19–21). However, we are unaware of any intervention studies with hazelnuts that have focused on examining micronutrient status in older adults.

Evaluation of micronutrient status can be difficult, especially the evaluation of vitamin E status (22, 23). Although circulating α-tocopherol concentrations are generally higher in adults aged >50 y than in younger adults (22), the increased prevalence of hypercholesterolemia in older adults (24) makes interpretation of circulating α-tocopherol concentrations difficult because abnormal cholesterol levels are a reflection of aberrant lipoprotein metabolism (23, 25). High plasma lipids increase the amount of α-tocopherol that is retained in the circulation (25). Thus, a reliance on plasma tocopherols without considering circulating lipids is problematic.

Previous studies on the ability of hazelnuts to increase vitamin E status in humans have reported mixed results, with smaller amounts of nuts less likely to significantly change plasma α-tocopherol concentrations (13, 26). To obviate this assessment solely by the use of circulating α-tocopherol concentrations, the urinary α- and γ-carboxyethyl hydroxyxychromanol (α- and γ-CEHC) are alternate biomarkers of α- and γ-tocopherol status that increase with vitamin E intake (27, 28). Low α-CEHC excretion is considered an indicator of poor α-tocopherol status, whereas high α-CEHC excretion is indicative of an α-tocopherol intake that exceeds the body’s needs (27, 29). However, this novel biomarker of α-tocopherol intake has not been widely used and has not been measured in humans consuming increased dietary vitamin E intakes, such as increased hazelnut consumption, to reinforce the validity of α-CEHC as a biomarker of vitamin E intakes.

The objective of this study was to determine the extent to which daily hazelnut consumption by healthy older adults for 16 wk improves biomarkers of vitamin E and magnesium. For a detailed assessment of vitamin E status, plasma α- and γ-tocopherol concentrations were determined together with urinary α- and γ-CEHC excretion. In addition, a lymphocyte proliferation assay was utilized to evaluate the status of several other micronutrients. Because hazelnut consumption is reported to decrease blood lipids and improve glucose homeostasis, these biomarkers were also monitored in the study.

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Abbreviations used: Block-FFQ, Block Brief Food Frequency Questionnaire; CEHC, carboxyethyl hydroxyxychromanol; EAR, Estimated Average Requirement; NS-FFQ, nut and seed FFQ.

Methods

Subjects

Of the 152 persons screened, 62 were invited for further screening, and 41 met the inclusion and exclusion criteria and were enrolled in the study (NCT0348589), which took place from June 2016 to September 2017. Of the enrolled participants, 32 (22 women, 10 men) completed the 16-wk intervention. To be eligible for the study, participants needed to be ≥55 y of age, in generally good health (fasting glucose <126 mg/dL, high-sensitivity C-reactive protein <10 mg/dL, LDL cholesterol <160 mg/dL, TGs <200 mg/dL), without gastrointestinal disorders or bariatric surgeries, and not consuming nuts frequently but were willing to eat hazelnuts daily for 16 wk. All subjects had to have a vitamin E intake level <10 mg α-tocopherol/d, as evaluated through the use of an FFQ (described below) and estimation of the consumption of vitamin E–rich foods. In addition, eligible participants must not have recently used (<1 y) vitamin E–containing dietary supplements and had to be willing to avoid all other dietary supplements except calcium and vitamin D during the course of the study. The study protocol was approved by the Institutional Review Board at Oregon State University. All participants provided written informed consent.

Hazelnuts

Hazelnuts (Corylus avellana L. “Barcelona”) were provided by the Hazelnut Marketing Board of Oregon. Although some nutritional characteristics of this nut cultivar grown in Oregon are known (30), a detailed compositional analysis is not yet available. The nuts used for this study were grown in Oregon’s Willamette Valley and provided in small serving size packets of dry roasted hazelnuts. Hazelnuts were evaluated for vitamin E content by HPLC (described below) several times during the study to ensure the average concentration of α- and γ-tocopherols were relatively unchanged between batches of nuts.

Study design

This study was designed to determine the effects of a 16-wk intervention of hazelnuts on vitamin E and magnesium status in a group of older men and women, and used a post-pre design without a control group to adjust for temporal changes. Subjects consumed ~57 g hazelnuts/d and were asked to refrain from eating all other nuts, seeds, and many vitamin E- and magnesium-rich food items. Participants were not required to make other dietary changes, but participants were informed of the calorie and fat content of the nuts (~350 kcal; 34 g fat).

At the start of the study (baseline) and at the end of the intervention (week 16), participants provided fasting blood (collected in vacutainers; Becton Dickinson) and spot urine samples after an overnight fast. Plasma was separated from blood by centrifugation and divided into aliquots. Both plasma and urine aliquots were kept frozen at −80°C until analysis.

Initially, participants were provided with sufficient hazelnuts to consume daily for 1 mo along with a simple diary to record their daily consumption. They were asked to return at weeks 4, 8, 12, and 16 to obtain additional nuts (except at week 16) and to return the diary. Anthropomorphic measurements (height, weight, and blood pressure) were performed at baseline and study end with the same study staff using the same scale, stadiometer, and sphygmomanometer for every participant.

Dietary assessment

Subjects completed 2 dietary questionnaires as part of the trial: the Block Brief Food Frequency Questionnaire (Block-FFQ) provided by NutritionQuest.com to survey general dietary patterns, as well as a nut and seed FFQ (NS-FFQ; Supplemental document 1) to provide greater detail on vitamin E intake habits. The Block-FFQ does not assess specific nuts consumed; therefore, we developed a small survey (NS-FFQ) specifically designed to assess nuts and other common sources of vitamin E (Supplemental Document 1). The NS-FFQ was based on the style of the Block-FFQ, but this questionnaire specifically focused on the vitamin E–rich foods common in the US diet, such as nuts/seeds, oils, avocados, vegetarian meat substitutes, and soybeans.
Biochemical analyses

Plasma and serum were provided to Clinical Laboratory Improvement Amendments–certified clinical laboratories for analysis. The complete metabolic panel and lipid profiles were obtained with the use of the ACE Excel autoanalyzer, insulin by the Tosoh 3600ALIA analyzer, and high-sensitivity C-reactive protein by nephelometry (Oregon State University’s Student Health Services). Magnesium analysis was performed with the use of a spectrophotometric assay (Beckman Coulter AU 5800 chemistry analyzer; Quest Diagnostics).

Vitamin E (α- and γ-tocopherol) concentrations in plasma or hazelnuts were quantified, as we described elsewhere (31). Briefly, samples (1 g of hazelnut kernel powder or 100 μL plasma) were extracted after saponification in alcoholic potassium hydroxide with 1% ascorbic acid, then the extracts were injected into an HPLC with a Synergi Hydro-RP column (Phenomenex) coupled to an ammonerometric electro-chemical detector (LC-4B ECD; Bioanalytical Systems Inc.). Tocopherols were identified and quantified with the use of authentic standards. Plasma tocopherol concentrations and values normalized to circulating lipids (total cholesterol + TGs) were calculated.

Urinary α- and γ-CEHCs were extracted via a modified method of Li et al. (32). Briefly, thawed urine samples were stabilized with ascorbic acid and acidified with HCl for 1 h at 60°C. An internal standard (trolox; Sigma-Aldrich) was added, then the extract was injected into an Acquity UPLC BEH C18 column with a Waters XEVO triple quadrupole mass spectrometer. Analytes were detected through the use of multiple reaction monitoring at the following transitions: α-CEHC (m/z 277/163), γ-CEHC (m/z 263/149), and trolox (m/z 249/163). Sample peaks were analyzed by comparison to authentic standard compounds and adjusted with the use of the internal standard. Urinary creatinine was measured with a kit (Diagnostic kit #555; Sigma-Aldrich). CEHCs are reported per gram of creatinine. This ratio does not vary by time of day (29), therefore, a casual urine sample was used for analysis.

Lymphocyte proliferation assay

An assessment of intracellular micronutrient levels, as described in detail by Mischley et al. (33), was performed by Spectracell Laboratories. Although the exact procedure is proprietary, product literature indicates that peripheral blood mononuclear cells are isolated from provided blood samples. The peripheral blood mononuclear cell proliferation rates after phytihemagglutinin stimulation were used as indicators of micronutrient status by comparing cells growing in a micronutrient-deficient medium with those in a control cell growth medium. Because only one micronutrient is absent in each test, changes in proliferation rate (expressed as percentage of control) are expected to correlate with cellular stores of that micronutrient. Glutathione depletion was determined by the proliferation rates observed after exposure of cells to buthionine sulfoximine, a glutathione synthesis inhibitor. Total antioxidant function was determined by incubation with cumene hydroperoxide, a source of lipid peroxidation.

Statistical analysis

Statistical analysis was performed with the use of Prism software (GraphPad Software) and statistical power analysis was performed with the use of SAS version 9.4 (SAS-Institute). The study was designed as a pre-post intervention study, with no untreated control group (34). There were 3 primary endpoints in this study, which were calculated as the mean changes in measurements between baseline and the end of the 16-wk intervention for 1) plasma α-tocopherol, 2) urinary α-CEHC (α-tocopherol metabolite), and 3) serum magnesium. Power calculations are based on our previous data (35) and conservative estimates were used for the SDs for plasma α-tocopherol (6.0 μM) and urinary α-CEHC (0.3 μM/g creatinine); that for serum magnesium (0.2 mg/dL) was based on literature data (36, 37). A correlation of r = 0.6 was used for each of the 3 outcomes. Based on these data and the hazelnut nutrient composition, we anticipated a change of α-tocopherol = 2 μM, α-CEHC = 0.81 μmol/g creatinine, and magnesium = 0.1 mg/dL. Using a 2-sided t test at the 0.05 significance level and n = 32 participants, we had >99% power to detect a significant change for α-CEHC, 86.5% power to detect a significant change for magnesium, and 53.5% power to detect a significant change for plasma α-tocopherol, which is a less sensitive indicator of dietary vitamin E consumption than is urinary α-CEHC (27).

Paired t tests were conducted to determine treatment differences. As a secondary analysis, we tested for effect modification by gender, age, BMI, baseline concentrations of plasma α-tocopherol and lipids, and dietary intake of vitamin E using 2-factor ANOVA with Tukey’s post hoc analysis. In addition, we used Pearson and Spearman correlation coefficients to evaluate the relationship between dietary intake amounts and endpoints, including those measured by the lymphocyte proliferation assay. All statistical comparisons were 2-sided with significance set at P ≤ 0.05. The results are reported as means ± SDs.

Results

Participants

A total of 152 individuals were screened initially for the study, of whom 41 met the eligibility criteria and were enrolled in the study (Supplemental Figure 1). Nine participants [7 women, mean ± SD (range) age: 69 ± 8 (60–82 y)] withdrew during the study (4 were unwilling to adhere to the study inclusion criteria; 2 were unable to eat the quantity of hazelnuts required; the 2 oldest participants withdrew for medical issues unrelated to the study; 1 moved out of state), leaving 32 participants for the final analysis, except as noted. As expected from an aging population, participants were overweight and had slightly elevated blood pressure, fasting serum glucose, total cholesterol, and LDL cholesterol (Table 1). Before the study start, 3 participants took statins, 4 took medications to lower blood pressure, and 20 (62.5%) took dietary supplements other than vitamin E (primarily vitamin D, calcium, or both, which they were allowed to continue).

Hazelnuet consumption

Analysis of the Oregon hazelnuts used in the study showed that the mean ± SD α-tocopherol concentrations were 15.4 ± 2.9 mg/100 g. Participants were requested to eat ~57 g hazelnuts/d, provided in sealed packets. Compliance was monitored by self-reporting and counts of unconsumed hazelnut packets. Overall, study participants were very compliant (range 87.9–100%; mean: 97%), the rough equivalent of increasing α-tocopherol intake by 7.7–10.4 mg/d. The estimated hazelnut consumption corresponds to ~130–160 mg α-tocopherol per day (Table 2).

TABLE 1 Lipid profile, glucose, insulin, and hs-CRP concentrations, blood pressure, and BMI of older adults at baseline and after 16 wk of daily consumption of ~57 g hazelnuts

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Baseline (µM)</th>
<th>Week 16 (µM)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>197 ± 33</td>
<td>193 ± 27</td>
<td>0.15</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>116 ± 28</td>
<td>109 ± 24</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>65 ± 15</td>
<td>67 ± 14</td>
<td>0.14</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>3.1 ± 0.7</td>
<td>3.0 ± 0.7</td>
<td>0.009</td>
</tr>
<tr>
<td>Plasma TGs, mg/dL</td>
<td>83 ± 30</td>
<td>86 ± 30</td>
<td>0.34</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>102 ± 7</td>
<td>99 ± 10</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum insulin, µU/mL</td>
<td>8.1 ± 7.9</td>
<td>8.3 ± 5.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Serum hs-CRP, mg/dL</td>
<td>1.2 ± 1.5</td>
<td>1.2 ± 1.4</td>
<td>0.83</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>120 ± 12</td>
<td>120 ± 12</td>
<td>0.07</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>76.6 ± 8.0</td>
<td>76.3 ± 7.8</td>
<td>0.78</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 ± 4.1</td>
<td>26.3 ± 4.3</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Values shown are means ± SDs, n = 32. BP: blood pressure; hs-CRP: high-sensitivity C-reactive protein.

Table 1: Lipid profile, glucose, insulin, and high-sensitivity C-reactive protein (hs-CRP) concentrations, blood pressure, and body mass index (BMI) of older adults at baseline and after 16 wk of daily consumption of ~57 g hazelnuts.
magnesium concentration is 1.6 mg/g [based on USDA food composition (10)], or an intake of ~93 mg magnesium/d.

Although dietary intakes were assessed by the Block-FFQ, the number of vitamin E-containing foods itemized in this FFQ is limited, especially with regard to nuts and seeds. The results of the Block-FFQ showed no change in energy, macronutrient, or micronutrient intake between baseline and week 16, despite the increased hazelnut consumption (Supplemental Table 1). When the vitamin E intakes from each of the assessment tools were compared with baseline plasma α-tocopherol concentrations, the outcomes from the NS-FFQ were significantly correlated (r = 0.40; P = 0.03), whereas the outcomes of the Block-FFQ were not (r = 0.12; P = 0.56), confirming our assumption about the sensitivity of the Block-FFQ to vitamin E intakes.

### Vitamin E status

To assess vitamin E adequacy in the study population, we measured urinary α- and γ-CEHC excretion. Urinary α-CEHC increased 33% from baseline to week 16 (P = 0.0006; Figure 1A), mirroring the increase in dietary α-tocopherol attributable to increased hazelnut consumption. Nonetheless, α-CEHC excretion remained relatively low, only reaching 1.14 ± 0.50 μmol/g creatinine after 16 wk. Urinary γ-CEHC concentrations remained unchanged after hazelnut consumption (Figure 1B).

The range of baseline plasma α-tocopherol concentrations (12.4–35.1 μmol/L) was unexpected based on the limited vitamin E intakes estimated from the Block-FFQ and suggested that some individuals regularly consumed sources of vitamin E that were not included in the FFQ. Neither plasma α- or γ-tocopherol concentrations changed significantly after the 16-wk intervention (Table 2). Further, no significant differences were detected when data were adjusted by age, BMI, gender, or serum lipids. The association between changes in α-CEHC and α-tocopherol concentrations was weak (r = 0.33; P = 0.07). However, there was a positive correlation between the changes in urinary α-CEHC and γ-CEHC excreted (r = 0.52; P = 0.003), suggesting increased metabolism of both α- and γ-tocopherol during the course of the study.

### Magnesium status

The other primary objective was to assess improvements in magnesium status. Serum magnesium concentrations increased significantly (P = 0.05) with hazelnut consumption, but the change was small, only +2.1% (Table 2). All participants had serum magnesium concentrations that remained within expected reference ranges [1.8–2.3 mg/dL (5)] before and after hazelnut consumption.

### Lipid profile and other clinical markers

Both plasma LDL cholesterol concentrations (−6.0%, P = 0.02) and the total:HDL cholesterol ratios (−4.5%, P = 0.009) decreased with hazelnut consumption (Table 1). This effect was observed whether or not statin users were included in the analysis. A total of 40% (13 of 32) of the participants had a >10% decline in LDL cholesterol; these changes did not correlate with any other findings from the study. Plasma TGs, total cholesterol, and HDL cholesterol did not change significantly with the hazelnut intervention.

Fasting plasma glucose concentrations decreased 3.4% (P = 0.03) with hazelnut consumption (Table 1); however, only a total of 22% (7 of 32) of the participants experienced a >10% decline in glucose concentrations. Changes in serum insulin, CRP, and blood pressure were nonsignificant. The average body weight increased significantly by 0.75 kg over the 16-wk experimental period; 3 participants (9%) had a >5% increase in body weight and 1 participant (1%) had a >5% decrease in body weight.

### Lymphocyte proliferation assay

A functional readout of cellular micronutrient levels was tested with the use of a lymphocyte proliferation assay. Few changes in the proliferative capacity of the isolated lymphocytes
were detected (Table 3). The exceptions were detections of response in panthenic acid– or cyanocobalamin–deprived cells. No changes were detected in the responses for any other nutrient, including vitamin E or magnesium. However, “Total antioxidant function” did increase, suggesting an increase in the peroxide resistance, i.e., antioxidant capacity, of these cells. Nonetheless, there were no observed correlations of plasma \( \alpha \)-tocopherol concentrations with any of the observed lymphocyte proliferation outcomes.

Discussion

Hazelnuts are an excellent source (20% of the daily value) of some micronutrients, especially vitamin E and magnesium. The addition of hazelnuts to the diet of older adults who normally consume few magnesium- or vitamin E–rich foods improves the dietary intake of these micronutrients, but our study emphasizes the challenges of assessing changes in micronutrient status in these individuals. After hazelnut consumption for 16 wk with confirmation of “excellent” compliance by the participants, serum magnesium concentrations increased by <10%. More marked changes were observed in urinary \( \alpha \)-CEHC concentrations, a biomarker of vitamin E metabolism. Although plasma \( \alpha \)-tocopherol concentrations did not significantly increase after hazelnut consumption, assessing vitamin E status by measuring plasma \( \alpha \)-tocopherol concentrations is complicated by age-related changes in cholesterol and lipoprotein metabolism. Further, dietary \( \alpha \)-tocopherol intakes (assessed by summing daily values from the Block-FFQ and the NS-FFQ) were significantly correlated with urinary \( \alpha \)-CEHC concentrations \((r = 0.28, P = 0.03)\), but not with plasma \( \alpha \)-tocopherol concentrations [whether adjusted for lipids \((r = 0.01, P = 0.92)\) or not \((r = 0.10, P = 0.45)\)]. Our findings support the hypothesis that urinary \( \alpha \)-CEHC excretion increases with even minor increases in dietary \( \alpha \)-tocopherol intake (10 mg as opposed to typical supplement pills containing 400 IU) \((27, 28)\). Thus, measures of urinary \( \alpha \)-CEHC excretion show promise as a useful indicator of adequacy of overall vitamin E status.

A critical factor for the success of a vitamin E intervention study is the enrollment of participants with a low habitual intake of dietary \( \alpha \)-tocopherol. Although the \( \alpha \)-tocopherol intake data obtained in our survey of nuts and seeds (NS-FFQ) accounted for ~40% of the observed variation in plasma \( \alpha \)-tocopherol concentrations at study start, these dietary outcomes did not ensure low plasma \( \alpha \)-tocopherol concentrations. However, urinary \( \alpha \)-CEHC concentrations were low at study start and increased in most subjects after hazelnut consumption. Therefore, urinary \( \alpha \)-CEHC might be able to replace dietary assessment as a noninvasive biomarker in the future. Not only could this marker be used to identify more appropriate study populations for vitamin E intervention, it might be used in lieu of plasma \( \alpha \)-tocopherol concentrations to measure any impact on vitamin E status.

Diets rich in nuts are associated with a reduction in the risk of cardiovascular disease or cancer, and risk of mortality from respiratory disease, type 2 diabetes, and infections \((19, 21, 38, 39)\), which are all leading health concerns for older adults. Many clinical trials evaluating hazelnuts have primarily focused on either healthy \((14, 18, 26)\), overweight \((40)\), or hypercholesterolemic individuals \((13, 16)\), with only a fraction of study populations \(\geq 50\) y of age. Our study focused on a cohort of older adults; although they were generally healthy, our clinical workup revealed the prevalence of elevated blood pressure, BMI, plasma glucose, LDL cholesterol, and total cholesterol concentrations often found in this older population. Increasing nut consumption in these individuals presents an opportunity for possible intervention before the diagnosis of chronic disease or need for pharmaceutical interventions. Although our results did not recapitulate previously described benefits to HDL cholesterol or total cholesterol, our study shows small improvements in serum total:HDLC ratio and LDL cholesterol concentrations, as has been observed in other trials \((13, 14, 16, 20, 26)\). These positive but small changes should be viewed in this context of an aging population without gross hyperlipidemia or hyperglycemia. The number of individuals in the “ideal” range of LDL cholesterol \(<100\) mg/dL) increased from 28% at study start to 44% by the end of the study. In addition, ~53% of the study participants had plasma glucose concentrations <100 mg/dL (in the “normal” category) by the end of the study, compared with 31% before the hazelnut intervention. Previous trials demonstrated no significant changes in body weight or BMI with increased nut consumption in younger adults \((41)\); therefore, the increased body weight \(+0.75\) kg over 16 wk) observed in this trial may be attributed to a decline in physical activity or metabolism that is more common with advanced age.

Limitations in our study include the small study size, relatively short duration (16 wk) of the intervention, and lack of a control group. For example, changes in plasma HDL cholesterol concentrations may have been evident over a longer duration, within a larger population, or in comparison with individuals not consuming the lipids or dietary fiber provided.
by the nuts. Changes in α-tocopherol may have been evident if plasma α-tocopherol or urinary α-CEHC concentrations had been used instead of dietary FFQs during the screening process to include only participants with low vitamin E status. However, finding healthy older individuals willing to give up all nuts and other vitamin E–rich foods while avoiding dietary supplements was a major limiting factor to recruitment. Other limitations of the current study include the absence of a younger adult category, so that the effects of age could be properly delineated. In addition, an assessment of erythrocyte magnesium concentrations might have provided some additional insights, because serum magnesium concentrations are considered a poor indicator of whole-body magnesium status (42).

The lymphocyte proliferation assay did not provide any greater clarity on the impact of hazelnut consumption on cellular micronutrient status. The only possible result related to vitamin E status from the lymphocyte proliferation assay was an increased resilience of cells to a cumene hydroperoxide challenge. Although it is tempting to attribute this change to increased cellular concentrations of α-tocopherol, the poor response by this assay to other micronutrients found in hazelnuts casts doubt on this finding.

In conclusion, our study adds to the growing body of knowledge supporting the benefits of hazelnut consumption. A large fraction of the US population has inadequate intakes of many minerals and vitamins, including vitamin E (2-4, 22)—especially older adults, who often have lower intakes of micronutrients from food sources (3, 4). Vitamin E is a so-called “shortfall” micronutrient [identified in the Dietary Guidelines for Americans (43) as a micronutrient which is frequently consumed at levels below the EAR (vitamin E: 12 mg/dl)]. Therefore, promoting good dietary sources of vitamin E is important. Despite the limitations of this study, the significant increase in the α-tocopherol metabolite α-CEHC indicates that hazelnut consumption can improve vitamin E status and supports the notion of increased hazelnut consumption as part of a healthy dietary pattern.

Acknowledgments
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References


