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A Walnut Diet Improves Endothelial Function in Hypercholesterolemic Subjects

A Randomized Crossover Trial

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Background—Epidemiological studies suggest that nut intake decreases coronary artery disease (CAD) risk. Nuts have a cholesterol-lowering effect that partly explains this benefit. Endothelial dysfunction is associated with CAD and its risk factors and is reversed by antioxidants and marine n-3 fatty acids. Walnuts are a rich source of both antioxidants and α-linolenic acid, a plant n-3 fatty acid.

Methods and Results—To test the hypothesis that walnut intake will reverse endothelial dysfunction, we randomized in a crossover design 21 hypercholesterolemic men and women to a cholesterol-lowering Mediterranean diet and a diet of similar energy and fat content in which walnuts replaced ≈32% of the energy from monounsaturated fat. Participants followed each diet for 4 weeks. After each intervention, we obtained fasting blood and performed ultrasound measurements of brachial artery vasomotor function. Eighteen subjects completing the protocol had suitable ultrasound studies. Compared with the Mediterranean diet, the walnut diet improved endothelium-dependent vasodilation and reduced levels of vascular cell adhesion molecule-1 ($P<0.05$ for both). Endothelium-independent vasodilation and levels of intercellular adhesion molecule-1, C-reactive protein, homocysteine, and oxidation biomarkers were similar after each diet. The walnut diet significantly reduced total cholesterol ($-4.4\pm7.4\%$) and LDL cholesterol ($-6.4\pm10.0\%$) ($P<0.05$ for both). Cholesterol reductions correlated with increases of both dietary α-linolenic acid and LDL γ-tocopherol content, and changes of endothelium-dependent vasodilation correlated with those of cholesterol-to-HDL ratios ($P<0.05$ for all).

Conclusions—Substituting walnuts for monounsaturated fat in a Mediterranean diet improves endothelium-dependent vasodilation in hypercholesterolemic subjects. This finding might explain the cardioprotective effect of nut intake beyond cholesterol lowering. **(*Circulation*. 2004;109:1609-1614.)**

Key Words: antioxidants ■ diet ■ endothelium ■ hypercholesterolemia ■ lipoproteins

Nuts are fatty foods rich in unsaturated fatty acids.[1] Epidemiological studies have shown that frequent nut consumption decreases the risk of coronary artery disease (CAD), with adjusted relative risk reductions approaching 50% for nut intakes of >4 to 5 servings per week compared with little or no intake.[2–4] Feeding trials have demonstrated that healthy diets enriched with a variety of nuts consistently reduce total and LDL cholesterol by 5% to 15%.[1,4–8] The lipid effects of nut intake only explain in part the CAD risk reduction observed in prospective studies, suggesting that nuts might have antiatherosclerotic effects beyond cholesterol lowering.

Besides having a favorable fatty acid profile, nuts are a rich source of bioactive compounds with potential benefit on CAD risk such as dietary fiber, folic acid, and antioxidants.[1] Nuts also contain sizeable amounts of L-arginine, the precursor amino acid of the endogenous vasodilator nitric oxide (NO).[9] Walnuts differ from all other nuts by a high content of α-linolenic acid (ALA), a vegetable n-3 fatty acid,[10] which might confer them additional antiatherogenic properties.[11]

Endothelial dysfunction, an early event in the development of vascular disease, is associated with atherosclerosis and its risk factors, including hypercholesterolemia.[12] Recent studies indicate that coronary endothelial dysfunction predicts future CAD events.[13,14] Endothelial function can be assessed noninvasively in the peripheral circulation,[15] and brachial artery ultrasound measures correlate with those of coronary endothelial function.[16] Vascular reactivity may be improved by dietary factors such as marine n-3 fatty acids, antioxidants, and L-arginine,[17,18] but whole foods rich in these compounds have not been investigated. To test the hypothesis that walnut intake would improve endothelial function in subjects with

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TABLE 1. Composition of the Baseline Diet and the Prescribed and Actually Observed Study Diets

		Control Diet		Walnut Diet		
Variable	Baseline Diet, Actual*	Prescribed	Actual*	Prescribed	Actual*	P†
Energy, kcal/d	1846±402	1600–2800	1955±315	1600–2800	1973±333	>0.1
Fat, % energy	33.7±2.6	33	33.2±1.0	33	33.0±1.1	>0.1
SFA	7.5±1.6	6	5.5±0.8	6	4.8±0.6	>0.1
MUFA	18.3±3.5	22	20.3±3.0	15	14.2±0.6	<0.001
PUFA	4.3±1.0	5	4.2±0.3	12	11.5±0.8	<0.001
Linoleic (C18:2n-6)	3.5±0.8	4.1	3.4±0.2	9.7	9.4±0.6	<0.001
α-Linolenic (C18:3n-3)	0.34±0.16	0.4	0.35±0.09	1.8	1.79±0.15	<0.001
Marine n-3 PUFA	0.19±0.22	0.2	0.18±0.11	0.2	0.17±0.10	>0.1
Protein, % energy	19.8±2.3	17	16.3±1.8	17	16.7±1.5	>0.1
Carbohydrates, % energy	44.6±5.4	50	49.1±3.6	50	48.9±3.7	>0.1
Cholesterol, mg/d	243±62	150	183±80	150	147±51	0.091
Soluble fiber, g/d	5.9±1.9	8	8.3±2.2	8	8.2±1.8	>0.1

*Mean±SD values estimated from 7-day food records during each diet period.
†Statistical significance for comparison between study diets by paired *t* test.

hypercholesterolemia, we performed a randomized, crossover feeding trial by substituting a walnut-enriched diet for a healthy Mediterranean-type diet for effects on brachial artery vasomotor function and circulating markers of endothelial activation.

Methods

Subjects

Twenty-one nonsmoking, asymptomatic men and women with moderate hypercholesterolemia attending the Lipid Clinic at the Hospital Clínic of Barcelona (Spain) and following a cholesterol-lowering diet were recruited into a protocol approved by the institutional review board, and all gave informed consent. Eligibility criteria were age 25 to 75 years (after menopause in women), serum LDL cholesterol ≥3.36 mmol/L (130 mg/dL), triglycerides ≤2.82 mmol/L, absence of chronic illnesses or secondary hypercholesterolemia, and no known allergy to nuts. None of the participants took vitamin supplements, hormone replacement therapy, or medications known to affect lipid metabolism. They were offered free walnuts but no monetary compensation.

Intervention

Before the study, the general recommendations of a Mediterranean-type, cholesterol-lowering diet were reinforced in all eligible subjects, and baseline data were collected after 4 weeks. The self-reported nutrient contents of the baseline diet showed good adherence to dietary advice (Table 1). The 4-week dietary equilibration period produced a mean change of 3.4±7.2% in total cholesterol level ($P=0.031$). Smaller, nonsignificant changes were observed for LDL cholesterol, HDL cholesterol, and triglycerides. After this period, participants were individually randomized in a crossover design between 2 diet sequences for 4-week periods: a control, Mediterranean-type diet and an isoenergetic diet enriched with walnuts. Twelve participants followed the control diet first for 4 weeks and then switched to the walnut diet for the ensuing 4 weeks; 9 subjects followed the same diets in reverse order. Because diet-induced lipoprotein changes stabilize in <4 weeks,[19] we did not incorporate a washout period between diets. Participants ate on their own, a reason why detailed information was provided to them and, if appropriate, to their partners. Table 1 shows the nutrient content of the prescribed diets. The diets were composed of natural foodstuffs. Vegetable products and fish were emphasized, and red and processed meats, whole-fat dairy products, and eggs were limited. The walnut diet was similar to the control diet, but walnuts partially replaced olive oil and other monounsaturated fatty acid (MUFA)–rich foods such as olives and avocados (no nuts other than walnuts in the walnut diet were allowed during the study). Prepackaged daily allowances of raw, shelled walnuts were provided daily in amounts varying from 40 to 65 g (equivalent to 8 to 13 walnuts), according to the participants' total energy intake. Walnuts were consumed as snacks or with meals in desserts or salads. In the walnut diet, walnuts contributed ≈18% of the total energy and replaced 32% of the energy obtained from MUFA in the control diet.

The walnuts used in the study were analyzed by standard methods in a reference laboratory (IRTA, Generalitat de Catalunya). The composition (per 100 g) of walnuts was 14 g protein (of which 18% arginine), 14 g carbohydrate, and 69 g fat. The fatty acid composition was 10.1% saturated fatty acids (SFA), 16.9% MUFA, 59.6% n-6 polyunsaturated fatty acid (PUFA; linoleic acid), and 13.4% n-3 PUFA (ALA). Vitamin E components (per 100 g) were 1.8 mg α-tocopherol and 155 mg γ-tocopherol.

Compliance was assessed from 7-day diet recalls. The diets were analyzed with the Food Processor, version 8.44, software (ESHA Research) adapted to nutrient databases of specific Mediterranean foods when appropriate. Because walnuts are a particularly rich source of γ-tocopherol, its serum level was measured as a biological marker of adherence to the walnut diet.

Laboratory Measurements

Fasting blood samples were obtained at baseline and at the end of each diet period. Except for immediate lipoprotein determinations, serum and EDTA plasma samples were stored at −80°C and analyzed at the end of the study. Cholesterol and triglycerides were measured with enzymatic procedures. HDL cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride. Apolipoproteins (Apo) Al and B and lipoprotein(a) were determined by use of turbidimetry. The cholesterol content of VLDL and LDL was measured after separation of lipoproteins by density gradient ultracentrifugation as described.[6] Frozen LDL aliquots, preserved in a 100-g/L sucrose solution, were stored at −80°C for use in copper-induced oxidizability studies and measurement of vitamin E content at the end of the study.[6] Analytes determined by subject in frozen samples of whole serum or plasma as appropriate were γ-tocopherol and malondialdehyde (MDA) by standard high-performance liquid chromatography methods, oxidized LDL by a monoclonal antibody-based immunoassay (Mercodia AB), folic acid by enzyme immunoassay, homocysteine by fluorescence polarization immunoassay, soluble intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) by standard ELISA from DRG Diagnostica (Palex Cormedica), and

high-sensitivity C-reactive protein (hsCRP) by particle-enhanced immunonephelometry. All analyses were done in duplicate.

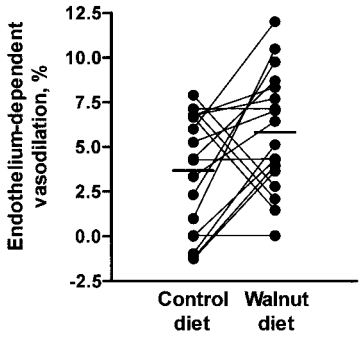
Endothelial Function

The noninvasive method of Celermajer et al[15] was used to evaluate endothelial function. Studies were performed at the end of each diet period. On the day of testing, participants ate a standard breakfast at 7 AM and then fasted until 1 PM, when a test meal was given. The meal consisted of a 100-g bread sandwich with 50 g lean pork ham, an apple, and tap water. For tests conducted during the control diet, the bread was soaked with 15 to 20 mL olive oil, whereas 20 to 32.5 g walnuts (half the daily dose) was incorporated into the test meal during the walnut diet. The varying doses of fat were adapted to individual energy requirements so that the test meal reproduce dietary conditions during the trial while keeping a constant amount of energy (550 to 650 kcal) and fat (19 to 27 g) across the 2 studies in each participant. Vascular ultrasound studies were performed 4 hours after ingestion of the test meal.

The studies were performed with a color Doppler Toshiba PowerVision ultrasound apparatus using multifrequency transducers (B-mode, 7.5 to 10 MHz; Doppler, 3.75 MHz). The brachial artery was imaged longitudinally, 2 to 5 cm above the antecubital crease, by an experienced ultrasound radiologist (I.N.) who was unaware of the stage of the experiment. An occluding cuff placed proximally on the forearm was inflated to a pressure of 300 mm Hg for 4.5 minutes and rapidly deflated to induce reactive hyperemia. Brachial artery scans were obtained continuously for 30 seconds before cuff inflation (first baseline), at 60 to 90 seconds after cuff deflation to assess endothelium-dependent vasodilation (EDV), for another 30 seconds after a 10-minute rest (second baseline), and 3 minutes after 0.4 mg sublingual glyceryl trinitrate to evaluate endothelium-independent vasodilation (EIDV). Arterial diameter was measured with ultrasonic calipers at end diastole, incident with the R wave on the ECG. Four cardiac cycles were analyzed for each scan, and measurements were averaged. Reproducibility of measurements over 1 month was documented in 15 healthy volunteers. Intraobserver reliability for measurement of EDV was 0.74 ($P=0.003$). The repeatability coefficient was 5.16, and the mean± SD difference between EDV values was 0.52±2.66%.

Statistical Analysis

For a crossover design, power calculations indicated that, to detect a mean absolute difference in EDV of 2% (SD, 2.66), 19 subjects would need to complete the study (α statistic, 0.05; power >0.9). Data are presented as mean±SD. Two-tailed *t* tests or the Wilcoxon rank-sum test for paired samples as appropriate was used to compare changes in outcome variables in response to diets. To examine whether there was any carryover effect from the order of diets for the 2-period crossover design, we performed a repeated-measures ANOVA with order and treatment as independent variables and outcome as the dependent variable. Differences between diets were tested also by ANCOVA using general lineal models, with baseline values or sex as covariates. Pearson's correlation coefficients were used to assess relationships between continuous variables. Analyses were performed with SPSS software (version 10.0).



EDV in brachial arteries of 18 men and women with moderate hypercholesterolemia after control Mediterranean diet and walnut diet given for 4 weeks each in crossover design. Bold lines represent mean values. Differences are statistically significant ($P=0.043$) by paired *t* test.

Results

Of the 21 participants randomly assigned to dietary intervention, 20 completed both study phases. One subject withdrew for personal reasons. The 8 men and 12 women who completed the trial had a mean age of 55 years (range, 26 to 75 years). The nutrient content of the self-reported diets was in good agreement with the planned diets (Table 1). The fatty acid composition of the control diet reflected the high MUFA content of olive oil, and that of the walnut diet mirrored the PUFA constituents of walnuts. According to participants' reports and recounts of empty packages, compliance with walnut ingestion was 100%. This was confirmed by the increase in the serum γ-tocopherol level during the walnut diet compared with the control diet (3.46±1.28 versus 1.89±1.05 μmol/L, $P<0.001$). Daily walnut consumption was well tolerated by all subjects.

Suitable brachial artery ultrasound measurements were available in 18 subjects. Compared with the olive oil–rich meal during the control diet, the walnut meal during the walnut diet significantly ($P=0.043$) improved EDV from 3.6±3.3% to 5.9±3.3%, a relative increase of 64% (the Figure and Table 2). Of the 18 participants, EDV improved by >2% in 9 subjects on the walnut diet. When these 9 diet-responsive subjects are compared with the 9 subjects who showed smaller changes or no benefit in response to the walnut diet, the only difference was percent change of the

TABLE 2. Vasomotor Function of the Brachial Artery and Soluble Markers of Endothelial Activation at Baseline and at the End of Each Diet Period

Variables	Baseline	Control Diet	Walnut Diet	*P**
Baseline artery diameter, mm	4.6±0.8	4.7±0.7	4.7±0.7	>0.1
EDV, %	3.4±3.7	3.6±3.3	5.9±3.3	0.043
EIDV, %	14.4±5.9	12.5±5.0	12.2±4.3	>0.1
Hyperemic flow, mL/min	265±89	312±105	287±112	>0.1
ICAM-1, μmol/L	355±99	370±89	343±72	>0.1
VCAM-1, μmol/L	474±177	465±229	378±149	0.045

Values are mean±SD.

*Statistical significance for comparison between experimental diets by paired *t* test.

TABLE 3. Body Weight, Blood Pressure, Serum Lipids, and Biomarkers of Oxidative Stress at Baseline and at the End of Each Diet Period

Variables	Baseline	Control Diet	Walnut Diet	P*
Body weight, kg	70.6±10.3	70.2±10.3	70.4±10.2	>0.1
Blood pressure, mm Hg				
Systolic	131±17	127±16	127±17	>0.1
Diastolic	80±9	75±10	77±9	>0.1
Cholesterol, mmol/L				
Total	6.93±0.70	6.72±0.51	6.43±0.69	0.017
LDL	4.75±0.62	4.64±0.46	4.33±0.47	0.010
HDL	1.61±0.41	1.59±0.40	1.57±0.44	>0.1
VLDL	0.57±0.31	0.49±0.33	0.53±0.38	>0.1
Triglycerides, mmol/L	1.39±0.59	1.32±0.64	1.43±0.77	>0.1
ApoAI, g/L	1.48±0.08	1.46±0.07	1.44±0.07	>0.1
ApoB, g/L	1.42±0.25	1.33±0.22	1.27±0.19	0.084
Ratios				
Total:HDL cholesterol	4.48±0.89	4.43±0.92	4.31±0.94	>0.1
LDL:HDL cholesterol	3.09±074	3.08±0.78	2.91±0.68	0.061
Lipoprotein(a), g/L	0.42 (0.51)	0.41 (0.37)	0.36 (0.37)	>0.1
Oxidation analytes				
LDL α-tocopherol, nmol/mg protein	12.8±3.0	12.6±2.2	11.2±2.2	0.007
LDL γ-tocopherol, nmol/mg protein	0.55±0.35	0.59±0.42	0.98±0.44	0.005
Lag time of LDL CD production, min	50.6±14.1	44.9±13.6	40.1±15.1	>0.1
Oxidized LDL, U/L	ND	48.5±9.4	45.9±8.4	>0.1
Malondialdehyde, nmol/L	110±28	123±42	106±32	0.086
Folic acid, ng/mL	5.3±3.1	5.8±3.5	5.8±3.3	>0.1
Homocysteine, μmol/L	9.8±4.7	9.7±2.8	9.9±3.0	>0.1
CRP, mg/dL	1.6 (2.1)	1.6 (2.3)	1.5 (3.8)	>0.1

CD indicated conjugated dienes. Values are mean±SD or medians (interquartile ranges). To convert cholesterol and triglycerides to mg/dL, multiply by 38.67 and 88.57, respectively.

*Statistical significance for comparison between the experimental diets by paired *t* test or the Wilcoxon rank-sum test.

cholesterol-to-HDL ratios (−5.9±7.9 versus 2.1±9.5; $P=0.070$). There was no evidence of a carryover effect between the periods. Adjustment for sex or baseline EDV did not change the results. At the end of each diet period, brachial ultrasound studies showed no significant differences in the baseline vessel diameter, EIDV, or hyperemic flow (Table 2). The level of VCAM-1 was significantly ($P=0.045$) lower during the walnut diet than during the Mediterranean diet. We did not find significant differences in ICAM-1 levels between the diets. There was an inverse correlation between changes in EDV and changes of cholesterol-to-HDL ratios ($r=-0.496$, $P=0.036$).

Table 3 shows the actual values at baseline and at the end of each diet period for other outcomes. Body weight and blood pressure were stable throughout the study. Compared with the Mediterranean diet, the walnut diet produced significant ($P<0.05$) reductions in total cholesterol (−4.4±7.4%) and LDL cholesterol (−6.4±10.0%). Parallel nonsignificant reductions in apoB and the LDL:HDL ratio were also observed with the walnut diet. The differences of effect between the 2 diets on the lipid profile did not change materially when adjusted for baseline values or gender. The changes between diets in total cholesterol, LDL cholesterol, and apoB were inversely correlated with both self-reported changes in dietary ALA as percent of energy ($r=-0.561$, $P=0.010$; $r=-0.578$, $P=0.008$; and $r=-0.594$, $P=0.006$, respectively) and changes in LDL γ-tocopherol ($r=-0.459$, $P=0.044$; $r=0.446$, $P=0.048$; and $r=-0.516$, $P=0.020$, respectively). The 2 diets had opposite effects on the LDL content of α-tocopherol and γ-tocopherol, whereas other biomarkers of oxidative stress and serum folic acid, homocysteine, and hsCRP were unaffected (Table 3).

Discussion

In this 8-week crossover feeding trial in subjects with moderate hypercholesterolemia, we found that substituting walnuts for ≈32% of the energy from MUFA in a cholesterol-lowering Mediterranean diet improves vascular endothelial function. Consistent with prior reports,[1,4–7] the walnut diet decreased total cholesterol and LDL cholesterol levels. Cholesterol reductions with the walnut diet were inversely related to self-reported dietary ALA increases. This was not unexpected, because cell culture experiments have shown that LDL enrichment with ALA following a walnut diet facilitates

receptor-mediated LDL clearance.[20] Our results provide direct evidence that regular walnut intake may reduce cardiovascular risk by a dual mechanism in subjects at risk for atherosclerosis.

One limitation of the study is the use of outpatient intervention diets as opposed to a controlled feeding trial with meals prepared at a metabolic kitchen. However, compliance was very good, and the actual diets consumed closely matched the prescribed diets (Table 1). In fact, the results show that motivated, free-living persons who have been given appropriate dietary advice may closely follow designed diets and incorporate substantial quantities of walnuts into their meals. Another limitation is that we evaluated postprandial endothelial function but did not obtain fasting measurements. Vascular reactivity may be impaired after a fatty meal, and this effect may be counteracted by n-3 fatty acids or antioxidants in the meals.[18] Nevertheless, the test meals were designed to reproduce the feeding conditions during the 2 dietary periods, and no other sources of variation of vascular reactivity were introduced. From this study, we cannot know whether, compared with an olive oil-rich meal and as a result of acute effects of dietary components, a walnut meal lessens the deterioration of EDV that presumably follows a fatty meal. However, because humans spend a good part of the day in a postprandial state, when the effects of foods on endothelial function are more likely to be relevant, it is arguable whether the chronic effect of the walnut diet can be separated from the repeated acute effects of walnut meals.

The walnut meal in a background walnut diet was associated with significant improvement in brachial artery EDV. The walnut diet also attenuated endothelial activation, as suggested by the reduction in VCAM-1 levels. The mechanism by which walnut intake may improve endothelial function remains uncertain. The inverse correlation between changes in EDV and those in cholesterol-to-HDL ratios suggests that the effect of the walnut diet may be mediated in part through an improved lipid profile. It is well established that hypercholesterolemia impairs EDV and that endothelial dysfunction can be reversed by aggressive cholesterol-lowering treatment.[12] Although significant, the average 6.4% decrease in LDL cholesterol observed with the walnut diet is modest compared with the profound LDL cholesterol reductions induced by hypolipidemic drugs or LDL apheresis that have been associated with improved EDV in clinical trials.[12] This suggests that other factors may play a role in the beneficial vascular effects of the walnut diet.

One component of walnuts that might favorably influence endothelial function is the plant n-3 fatty acid ALA. The walnut allowances used in the study provided daily amounts of ALA ranging from 3.7 to 6.0 g. These doses of ALA are nearly double those used in the intervention groups of 3 secondary prevention trials showing marked reductions in cardiac end points that have been attributed in part to supplemental ALA.[21–23] Intake of 20 g/d ALA from flaxseed oil for 1 month has been reported to improve arterial compliance despite increased LDL oxidation,[24] but no formal endothelial function studies have been performed after ALA supplementation. On the other hand, fish oil supplements at doses ranging from 3 to 10 g/d consistently ameliorate EDV in clinical studies.[18,25,26] This beneficial effect of marine n-3 fatty acids might be mediated by increased membrane fluidity of endothelial cells promoting enhanced synthesis and/or release of NO.[27] There is also in vitro evidence of reduced endothelial expression of VCAM-1 by marine n-3 fatty acids,[28] but fish oil intake has been associated with increased VCAM-1 levels, perhaps because of an enhanced oxidative stress.[17]

Despite its high PUFA content, the walnut diet had no deleterious effects on biomarkers of oxidative stress. Both the resistance of LDL to in vitro oxidation and the level of oxidized LDL were similar after the 2 diets, whereas the plasma level of MDA, an end product of the peroxidation process, decreased nonsignificantly after the walnut diet. Although there were predictable,[29] reciprocal changes in the LDL content of α- and γ-tocopherol between the 2 diets, the data suggest that endogenous antioxidants were spared during the walnut diet. Phenolic compounds in walnuts probably counteracted the pro-oxidant effects of PUFA on LDL.[30] These results provide further evidence that walnut diets are not associated with lipid peroxidation.[6,7] A recent report indicates that, among edible plants, walnuts have one of the highest contents of total antioxidants.[31] Oxidation markers were not worsened by the walnut diet, but neither were they improved; hence, our study does not support the antioxidant potency of walnuts as an alternative explanation for the observed beneficial effects on vascular reactivity.

γ-Tocopherol is another strong antioxidant that is particularly abundant in walnuts. The walnut diet provided 90 to 135 mg/d of this vitamin E component and increased its serum level $\approx$2-fold. Although γ-tocopherol has been investigated much less than α-tocopherol, it is increasingly recognized as a relevant antiatherogenic molecule.[32] The lack of clinical trials using γ-tocopherol for effects on markers of cardiovascular risk precludes any conclusions on its eventual vasomotor effects. Interestingly, serum cholesterol reductions were related to increases in the γ-tocopherol content of LDL. A likely explanation is that LDL γ-tocopherol is a marker of the bioavailability of the walnut constituents responsible for lowering cholesterol.

As the substrate for endothelium-derived NO, the amino acid L-arginine, still another important constituent of nuts,[9] also might have a positive effect on EDV. Walnut intake increased dietary L-arginine by 0.9 to 1.4 g/d. Endothelial function improves after treatment with L-arginine supplements starting at doses of 2 g/d, particularly in subjects with impaired NO synthesis such as patients with hypercholesterolemia or prior congestive heart disease.[17,18,33]

Endothelial dysfunction associated with cardiovascular risk factors or established congestive heart disease may be restored by various food components in the absence of any cholesterol lowering. Thus, supplements of marine n-3 fatty acids, L-arginine, folic acid, and antioxidant vitamins C and E and beverages rich in phenolic compounds such as red wine, fruit juice, and tea have demonstrated a beneficial vasomotor effect in various arterial beds.[18] Replacement of a diet rich in SFA with 2 cholesterol-lowering diets, a low-fat diet or an olive oil–rich, Mediterranean-type diet, has also been shown to improve brachial artery EDV in hypercholesterolemic

men.[34] This argues against the partial replacement of olive oil and other MUFA-rich foods in the walnut diet as being relevant to improved EDV. The unique composition of walnuts, a whole food rich in several nutrients and phytochemicals with the capacity to improve vascular reactivity, probably explains why substituting walnuts for part of the MUFA in a Mediterranean diet was associated with improved endothelial function in men and women with hypercholesterolemia. This finding might explain the cardioprotective effect of regular nut intake beyond cholesterol lowering. It also provides further support for the inclusion of walnuts in healthy diets.

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