Nuts and novel biomarkers of cardiovascular disease[1–4]

Emilio Ros

ABSTRACT

Nuts are energy-dense foods, rich in total fat and unsaturated fatty acids. The favorable fatty acid profile probably contributes to the beneficial effects of nut consumption observed in epidemiologic studies (prevention of coronary heart disease and diabetes) and feeding trials (cholesterol lowering). Besides fat, the complex matrices of nuts contain many bioactive compounds: vegetable protein, fiber, minerals, tocopherols, and phenolic compounds. By virtue of their unique composition, nuts are likely to benefit newer cardiovascular risk biomarkers, such as LDL oxidizability, soluble inflammatory molecules, and endothelial dysfunction. Protection of LDL oxidation by nut intake has been documented in some, but not all, clinical studies. In one study, feeding one daily serving of mixed nuts was associated with lower oxidized LDL concentrations. Regarding inflammation, cross-sectional studies have shown that nut consumption is associated with lower concentrations of circulating inflammatory molecules and higher plasma adiponectin, a potent antiinflammatory adipokine. Clinical studies with nuts have documented reduced inflammatory cytokine concentrations but no consistent changes of C-reactive protein. Only walnuts have been formally tested for effects on endothelial function. After both walnut diets and single walnut meals, favorable vasoreactivity changes have been observed. Walnut consumption also reduced expression of endothelin 1, a potent endothelial activator, in an animal model of accelerated atherosclerosis. Beneficial effects on vascular reactivity may be ascribed to several constituents of walnuts: L-arginine, the precursor of nitric oxide, α-linolenic acid, and phenolic antioxidants. Although more studies are warranted, the emerging picture is that nut consumption beneficially influences cardiovascular risk beyond cholesterol lowering. *Am J Clin Nutr* 2009;89(suppl):1649S–56S.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading causes of disability and death in industrialized nations and much of the developing world. Over the past 3 decades it has become clear that the initiation and progression of atherosclerosis, the pathological basis of CVD, results from a combination of abnormalities in lipoprotein metabolism, oxidative stress, chronic inflammation, and susceptibility to thrombosis (1). The prevailing theory of atherosclerosis formation postulates that endothelial injury by classical CVD risk factors (smoking, high blood pressure, elevated blood cholesterol) leads to trapping of LDL cholesterol within the arterial wall, which becomes oxidized, further activating the endothelium to secrete cytokines that attract circulating mononuclear cells expressing receptors

for these cytokines and initiate a self-perpetuating inflammatory process (1–3). As our understanding of the complex pathophysiology of atherosclerotic disease has evolved beyond the accumulation of cholesterol in the arterial wall, a series of circulating, functional, structural, and genomic biological markers that reflect arterial vulnerability have been proposed as potential novel risk factors for the development of CVD (4). Among them, biomarkers of oxidation (5), inflammation (6), and endothelial dysfunction (7) have received increasing attention.

Diet changes remain the cornerstone for prevention and treatment of CVD. The landmark Seven Countries Study (8) showed ecological associations between the saturated fatty acid (SFA) and cholesterol content of the diet and an increase of both serum cholesterol concentrations and the risk of coronary heart disease in cohorts of different countries. Since then, our understanding of how dietary factors modulate multiple CVD risk factors has increased substantially (9). For many years, dietary effects on plasma lipids have been at the center of the diet-heart hypothesis, but more evidence has accumulated that foods, nutrients, and dietary patterns may also influence novel atherogenic markers (10–15).

Tree nuts and peanuts (henceforth collectively referred to as nuts) are among the whole foods with a more impressive body of scientific evidence supporting beneficial effects on cardiovascular health. Large epidemiologic studies have consistently associated frequent nut consumption with a reduced incidence of coronary heart disease (16), whereas many feeding trials have clearly shown that intake of all kinds of nuts has a cholesterol-lowering effect, even in the context of healthy diets (17, 18). In epidemiologic studies and nut feeding trials, effects on novel biomarkers of CVD have been much less investigated than those

on incident coronary heart disease or the lipid profile. However, the emerging picture is that frequent nut consumption beneficially affects cardiovascular risk beyond cholesterol lowering. This article aims to review the epidemiologic and clinical trial evidence relating nut consumption to the novel CVD biomarkers of oxidation, inflammation, and endothelial dysfunction and to discuss possible mechanisms for the results reported. To put these findings in perspective, it is instructive to briefly review nut composition.

NUTRIENT CONTENT OF NUTS

Nuts are nutrient-dense foods because they have a high total fat content, ranging from 46% in cashews and pistachios to 76% in macadamia nuts, and provide 20–30 kJ/g nut (**Table 1**). Indeed, nuts are one of the natural plant foods richest in fat after vegetable oils. However, the fatty acid composition of nuts is not harmful because the SFA content is low (range: 4–16%). Nearly one-half of the total fat is made up of unsaturated fat, which includes monounsaturated fatty acids (MUFAs; oleic acid) in most nuts and mostly polyunsaturated fatty acids (PUFAs) including linoleic acid and α-linolenic acid (ALA; 18:3n–3), the plant n–3 (omega-3) fatty acid, in walnuts (19). It should be underlined that, with ≈3 g/serving, walnuts are the whole food with the highest content of ALA of all edible plants (20). The fatty fraction of nuts also contains sizeable amounts of plant sterols, with antioxidant (21) and cholesterol-lowering (22) properties.

Nuts are rich sources of other bioactive macronutrients. They are an excellent source of protein (≈25% of energy) and often have a high content of L-arginine, the amino acid precursor of the endogenous vasodilator nitric oxide (23). Nuts are also a good source of dietary fiber, which ranges from 4 to 11 g/100 g (Table 1), and in standard servings provide 5–10% of daily fiber requirements.

Among nut constituents there are also significant micronutrients. Nuts contain sizeable amounts of folate (Table 1) (22) and are rich sources of antioxidant vitamins (eg, tocopherols) and phenolic compounds (24). Almonds in particular are especially rich in α-tocopherol, whereas walnuts contain significant amounts of its isomer γ-tocopherol, increasingly recognized as a relevant antiatherogenic molecule (25). Importantly, most phenolics are located in the outer pellicle of nuts, which means that the peeled product loses much of its antioxidant capacity (24). Industrial bleaching, sometimes used to restore a desirable white color to the hard shells of nuts, destroys most of the antioxidants when the shells are naturally cracked, as shown for pistachios (26). These facts, rarely taken into consideration in prior studies with nuts, should not be overlooked in future feeding trials or when giving advice on nut intake in healthy diets.

Compared with other common foodstuffs, nuts have an optimal nutritional density in salutary minerals, such as calcium, magnesium, and potassium (Table 1). Like that of most vegetables, the sodium content of nuts is very low, ranging from undetectable in hazelnuts and pecans to 18 mg/100 g in peanuts (22). Obviously, this advantage of nuts is lost if they are consumed as a salted product.

In summary, whole, unpeeled, and otherwise unprocessed nuts have a unique composition, wherein most known nutrient and nonnutrient constituents are bioactive molecules. Most nut constituents have shown beneficial effects when clinically tested, in isolation or as part of enriched foods, for effects on diverse cardiovascular outcomes, including novel risk markers (10–13).

EFFECT OF NUTS ON NOVEL CARDIOVASCULAR BIOMARKERS

Various circulating or functional biomarkers of arterial vulnerability were primary or secondary outcomes in several controlled feeding trials of nuts. Soluble CVD biomarkers were also investigated in 3 recent epidemiologic studies in relation to frequency of nut consumption.

Nut consumption and inflammatory markers in epidemiologic studies

In a cross-sectional analysis of data from ≈6000 participants in the prospective Multi-Ethnic Study of Atherosclerosis (27),

TABLE 1
Average nutrient composition of nuts (per 100 g)[1]

Nuts	Energy	Total fat	SFA	MUFA	PUFA	Plant sterols	Total protein	Arginine	Fiber	Folate	α-Tocopherol	Calcium	Magnesium	Potassium
	kJ	*g*	*g*	*g*	*g*	*mg*	*g*	*g*	*g*	*μg*	*mg*	*mg*	*mg*	*mg*
Almonds	2418	50.6	3.9	32.2	12.2	120	21.3	2.47	8.8	29	25.9	248	275	728
Brazil nuts	2743	66.4	15.1	24.5	20.6	NR	14.3	2.15	8.5	22	5.7	160	376	659
Cashews	2314	46.4	9.2	27.3	7.8	158	18.2	2.12	5.9	25	0.9	37	292	660
Hazelnuts	2629	60.8	4.5	45.7	7.9	96	15.0	2.21	10.4	113	15.0	114	163	680
Macadamias	3004	75.8	12.1	58.9	1.5	116	7.9	1.40	6.0	11	0.5	85	130	368
Peanuts (dry roasted)	2448	49.7	6.9	24.6	15.7	NR	23.7	3.1	8.0	145	6.9	54	176	658
Pecans	2889	72.0	6.2	40.8	21.6	102	9.2	1.18	8.4	22	1.4	70	121	410
Pine nuts (dried)	2816	68.4	4.9	18.8	34.1	141	13.7	2.41	3.7	34	9.3	16	251	597
Pistachios	2332	44.4	5.4	23.3	13.5	214	20.6	2.03	9.0	51	2.3	107	121	1025
Walnuts	2738	65.2	6.1	8.9	47.2	72	15.2	2.28	6.4	98	0.7	98	158	441

[1] Data are from raw nuts, except when specified. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; NR, not reported. Data are from US Department of Agriculture Nutrient Database. Available from: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl (cited 2 May 2008).

concentrations of soluble inflammatory markers decreased across increasing categories of nut and seed consumption. After adjustment for values of all possible confounders measured except body mass index (BMI; in kg/m^2), mean concentrations of high-sensitivity C-reactive protein (CRP), an accepted measure of systemic low-grade inflammation, decreased from 1.97 mg/L in subjects who rarely or never ate nuts and seeds to 1.71 mg/L in subjects consuming them $\geq$5 times/wk (P for trend: 0.003). Corresponding adjusted mean concentrations of interleukin-6 (IL-6), a potent proinflammatory cytokine, decreased from 1.25 to 1.14 pg/mL (P for trend: 0.003). Adjusted fibrinogen concentrations decreased in a similar way from 343 to 331 mg/dL (P for trend: 0.003). However, further adjustment for BMI, a strong determinant of concentrations of inflammatory markers, attenuated the magnitude of the associations, yielding borderline statistical significance. Qualitatively similar associations were observed for nuts, seeds, and peanuts and peanut butter.

In another cross-sectional evaluation of 987 diabetic women from the prospective Nurses' Health Study (28), greater adherence to a Mediterranean dietary pattern was associated with higher concentrations of plasma adiponectin, a potent antiinflammatory cytokine originating from adipose tissue. Among the components of the Mediterranean diet, alcohol, whole grains, and nuts showed the strongest associations. The age- and energy-adjusted mean adiponectin concentration increased from 5.10 μg/mL in the lowest quintile of nut consumption to 6.15 μg/mL in the upper quintile (P for trend < 0.01), but further adjustment for additional covariates, including BMI, again attenuated the strength of the association, which became of borderline significance (P = 0.08).

A third cross-sectional study was performed in 772 older subjects at high risk of CVD living in Spain with the purpose of assessing adherence to the Mediterranean dietary pattern and its food components in relation to concentrations of soluble inflammatory markers (29). Adjusted mean serum concentrations of intercellular adhesion molecule 1 (ICAM-1; P for trend: 0.003) and vascular cell adhesion molecule 1 (VCAM-1; P for trend: 0.08), but not those of CRP or IL-6, decreased across increasing tertiles of nut consumption. ICAM-1 and VCAM-1 are cytokines signaling for cell recruitment to the arterial wall, and their circulating concentrations reflect the degree of endothelial activation by CVD risk factors (3).

Effects of nut diets on novel cardiovascular biomarkers in clinical studies

Biomarkers of oxidation

Because a substantial fraction of the fat contained in most nuts is made up of MUFAs, which are not an oxidation substrate, enrichment of lipoprotein lipids with these fatty acids after nut intake might decrease their susceptibility to oxidation. Conversely, detrimental changes of lipoprotein oxidation might occur after the consumption of walnuts because they contain high amounts of PUFAs, and double bonds in these fatty acids are preferred initiation sites for oxidative processes (30).

Oxidation markers after feeding MUFA-rich nuts have been examined in several clinical trials. Results have not been entirely consistent in studies involving almonds. In a study of healthy subjects, Berry et al (31) showed subjects were less prone to oxidation of plasma and LDL lipids after an almond diet than

after a low-fat diet. Jenkins et al (32, 33) compared diets supplemented with 2 almond doses with a similar diet supplemented with muffins in hypercholesterolemic subjects. In the first report of that study (32), the almond diets were associated with significant reductions of conjugated dienes (a marker of lipid oxidation) in LDL cholesterol by comparison with the muffin diet. In another report (33), lower concentrations of plasma malondialdehyde (an overall measure of lipid oxidation) (33) and urinary F$_2$-isoprostanes (a family of eicosanoids produced by nonenzymatic free radical oxidation of arachidonic acid) were observed after the almond diets. In a short-term crossover study by the same investigators (34), almond meals were associated with increased postprandial serum thiol concentrations (an inverse measure of protein oxidation) compared with bread, rice, or potato meals. However, Hyson et al (35) failed to show any improvement in the susceptibility of LDL cholesterol concentrations to an oxidative stress after feeding either whole almonds or almond oil to healthy individuals, even though SFA intake was lower during the almond diets. Single-feeding trials with diets enriched in hazelnuts (36), pistachios (37), and macadamia nuts (38) have shown an improved oxidation status, but the results must be interpreted with caution because in these studies the SFA content of the nut diets was lower than that of the comparator diets. Diets enriched in peanut products also reduced the susceptibility of LDL cholesterol to oxidative stress compared with an average American diet rich in fat, but not when compared with a low-fat diet of similar SFA content (39).

The ex vivo resistance of LDL cholesterol to oxidation was a secondary endpoint in 3 crossover feeding trials comparing PUFA-rich walnut diets with other healthy diets matched for SFA content for effects on cardiovascular risk markers, and none of them found between-diet differences (40–42). In one study (42), the walnut diet was associated with nonsignificant decreases of the serum concentrations of malondialdehyde and oxidized LDL (oxLDL), a more atherogenic particle than native LDL, by 14% and 5%, respectively. In a recent parallel feeding trial in patients with metabolic syndrome (43), no changes of the serum oxidation profile were observed after cashew, walnut, or control diets. In another parallel feeding trial with higher statistical power than usual clinical studies with nuts, the Prevención con Dieta Mediterránea (PREDIMED) study (44), a Mediterranean diet enriched with 30 g raw, unpeeled, mixed nuts (half of them walnuts), given for 3 mo to older subjects at high cardiovascular risk, was associated with a significant 10% reduction in circulating oxLDL concentrations. A recent crossover study examined postprandial oxidation after consumption of a high-fat, high-SFA meal enriched with walnuts or olive oil in healthy subjects and patients with moderate hypercholesterolemia (45). Neither meal affected the ex vivo susceptibility of LDL cholesterol to an oxidative challenge or circulating oxLDL concentrations in any of the 2 study groups. In summary, the available evidence suggests that MUFA-rich nuts moderately improve oxidative status, whereas PUFA-rich nuts (walnuts) have a neutral or slightly beneficial effect.

Biomarkers of inflammation

Plasma CRP concentrations were a secondary endpoint in 3 controlled clinical studies investigating nut diets for effects on lipid profiles (32, 42, 46). Two studies were crossover trials in

hypercholesterolemic subjects who were given 2 doses of al-monds (32) and single doses of walnuts that contained ≈18% of energy (42), respectively, and neither study found significant CRP changes. In one study, however, serum inflammatory cy-tokines ICAM-1 and VCAM-1 were measured, and the latter was significantly reduced by 19% after the walnut diet compared with the control diet (42). A third study had a parallel design and was conducted in patients with metabolic syndrome with 3 diets (walnuts, cashews, and control) and showed a significant in-crease of CRP with the walnut diet and no change with the cashew diet (46). Because CRP also increased after the control diet, the elevation associated with the walnut diet was suggested to be nonspecific. In addition, contrary to usual findings, none of the 2 nut diets was associated with a cholesterol-lowering effect (46). The more statistically powered PREDIMED trial also failed to show any effect of a Mediterranean diet enriched with 30 g mixed nuts on circulating CRP concentrations (47). How-ever, the plasma concentration of the inflammatory mediators IL-6, ICAM-1, and VCAM-1 decreased after the Mediterranean diet with nuts compared with the control diet (47). In the acute study of Cortés et al (45), postprandial rises of inflammation markers were similarly blunted after olive oil– and walnut-enriched meals, except for soluble E-selectin, another potent proinflammatory cytokine, which was lower after the walnut meal than after the olive oil meal.

Recent studies by Zhao et al (48, 49) used walnuts and walnut oil to enrich the diet in PUFAs and, particularly, ALA and to compare effects on inflammatory markers (48) and proin-flammatory cytokine production by blood mononuclear cells (49) with those of an average American diet. Decreased in-flammation was observed in both studies, but the beneficial changes might have been due to SFA replacement rather than to ALA in walnut products.

In summary, nut consumption appears to have no effect on CRP, but it is associated with reduced concentrations of other soluble inflammatory biomarkers. Clearly, more studies are needed on this important subject.

Endothelial dysfunction

Walnuts are the only nuts that have been formally studied for effects on vascular reactivity, as assessed by brachial artery va-sodilation after ischemic occlusion of the forearm (7). A recent crossover feeding trial tested the hypothesis that, when fed for 4 wk each, a walnut-supplemented diet would improve endothe-lial function in 21 patients with hypercholesterolemia compared with an isoenergetic healthy Mediterranean diet (42). The results showed that, by comparison with the control diet, which had a similar SFA content, the diet supplemented with walnuts (con-taining ≈18% of energy) attenuated the endothelial dysfunction associated with the abnormal lipid profile. Of note, in that study changes in endothelium-dependent vasodilation correlated in-versely with those of total-to-HDL cholesterol ratios ($r = 0.496$, $P = 0.036$) but not with changes of total or LDL cholesterol. In a complementary study in healthy subjects and patients with hy-percholesterolemia, which also had a crossover design, Cortés et al (45) showed that adding walnuts to a high-fat, high-SFA meal counteracted ensuing postprandial endothelial dysfunction by comparison with eating the same meal supplemented with olive oil.

In summary, the results of those 2 studies suggest that walnut consumption has both acute and chronic beneficial effects on vascular reactivity. Further studies are needed to confirm that nut intake influences endothelial function.

Possible mechanisms for the effects of nuts on novel cardiovascular biomarkers

The unique composition of nuts, a natural food rich in many nutrients and phytochemicals with the capacity to improve car-diovascular outcomes, probably explains why nut-supplemented diets tend to be associated with favorable changes of CVD biomarkers.

First, regarding oxidation, nuts possess remarkable antioxidant potential, derived from tocopherols, abundant phenolic com-pounds (24), phytosterols (21), and, possibly, melatonin, which was shown to be present in substantial amounts in walnuts, providing a significant antioxidant load in an experimental rat model (50). Besides the classical antioxidants, characterized by their ability to directly scavenge or neutralize reactive oxidant species, nuts also contribute to building endogenous antioxidant defenses. This is the case for Brazil nuts, which are rich natural sources of selenium, a critical component of selenoproteins active in antioxidant reactions, among other important physio-logic functions (51). As recently reported, consuming as little as 2 Brazil nuts/d suffices to improve selenium status and to in-crease the activity of whole-blood glutathione peroxidase ac-tivity, an endogenous antioxidant enzyme (52). β-Sitosterol, the predominant plant sterol in nuts (22), has also been shown to induce antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase (21). Furthermore, the various bioavail-able antioxidants present in nuts and other edible plants work in concert to upgrade the complex antioxidant network necessary to sustain cellular function (10). Thus, polyphenols cooperate with vitamins C and E (53) and β-sitosterol (54), and α-tocopherol synergizes with γ-tocopherol (55), to together increase antioxi-dant capacity higher than that provided by each separate com-pound.

The fatty acid components of nuts may differently influence oxidation processes, however, and this needs to be considered for synergy or opposition to effects of constituent antioxidants. Thus, nuts rich in MUFAs, a fatty acid resistant to oxidative stress, could be expected to favorably influence oxidation biomarkers in clinical studies, whereas nuts in which oxidation-prone PUFAs predominate, such as walnuts, could enhance oxidation unless counterbalanced by an increased load of antioxidants. As de-scribed, both an antioxidant effect of MUFA-rich almonds and a neutral effect of walnuts have been observed in clinical studies. That the PUFAs in walnuts do not increase oxidative potential is not surprising given that they are among the edible plant foods disclosing highest total antioxidant capacity, by far surpassing that of other nuts (24, 56). Whole, unpeeled nuts should be consumed for maximum antioxidant potential, which resides in the outer pellicle (24). This might explain why in the clinical study of Hyson et al (35) almond oil lacked antioxidant power, but it does not explain why whole almonds behaved similarly, unless they had been processed and lost their skins (the charac-teristics of the almonds given were not described in the report).

Second, some nut components have shown antiinflammatory properties when tested in isolation in clinical and experimental

studies, which may explain why similar effects are observed in epidemiologic and clinical studies with nuts. Thus, there is growing evidence that dietary polyphenols in nuts, fruit, cocoa, tea, and wine may have antiinflammatory effects, mediated by both their antioxidant action and modulation of signal transduction pathways, such as the nuclear transcription factor κB, with ensuing down-regulation of inflammatory genes in endothelial cells and macrophages (57). Walnuts are particularly rich in a specific phenolic compound, ellagic acid, which has shown potent antiinflammatory effects in experimental studies (58, 59). Antioxidant tocopherols, abundant in nuts, are also believed to have antiinflammatory effects (60).

As discussed, walnuts are the only nuts that contain substantial amounts of ALA. By analogy with marine n–3 PUFAs, ALA could be expected to show antiinflammatory properties, but this has been assessed in relatively few studies and only modest or nil effects have been reported (61). Interestingly, in a study in which human monocytic cells (THP-1) were cultured with different fatty acids in the presence of lipopolysaccharide, secretion of IL-6, IL-1β, and tumor necrosis factor-α, all potent inflammatory cytokines, were significantly decreased after treatment with PUFAs compared with palmitic acid, and ALA was one of the PUFAs eliciting more favorable effects (62). Those findings suggest that ALA in walnuts might induce an antiinflammatory response. Nevertheless, most feeding studies examining the effects of ALA on inflammatory outcomes have used flaxseed oil, which is particularly rich in this fatty acid but is devoid of the many other bioactive compounds found in whole walnuts.

Magnesium, a mineral found in nuts at higher amounts than in any other edible plants (22), could contribute to the reduction of inflammatory biomarkers observed in feeding studies with nuts. Magnesium deficiency has been hypothesized to be a factor in the pathogenesis of insulin resistance, type 2 diabetes, hypertension, and CVD, conditions with a common background of chronic inflammation (63). Three large cross-sectional studies have reported an inverse association of dietary magnesium intake with the plasma concentrations of inflammatory markers CRP (64–66), E-selectin and ICAM-1 (66). Although the underlying mechanism remains to be elucidated, the likely explanation for these antiinflammatory effects is the causal link between magnesium homeostasis and insulin resistance. Thus, together with other antiinflammatory components, the magnesium content of nuts might be relevant to the reduction of both incident diabetes (67) and prevalent metabolic syndrome (68) observed in association with nut consumption.

Finally, several nut constituents might favorably influence endothelial activation and vasomotor tone. Endothelial dysfunction is a critical event in atherogenesis that is implicated both in early disease and in advanced atherosclerosis, whereby it relates to perfusion abnormalities and the causation of ischemic events (1, 2, 7). It is characterized by a decreased bioavailability of nitric oxide, the endogenous vasodilator synthesized from L-arginine (23), and increased expression of proinflammatory cytokines and cellular adhesion molecules. Endothelial injury caused by cardiovascular risk factors or atherosclerotic vascular disease reduces nitric oxide production, and this is followed by arterial wall abnormalities, both functional (inhibition of vasodilatation or paradoxical vasoconstriction) and structural (smooth muscle cell growth and blood cell adhesion) that are responsible for the initiation, development, and progression of atherosclerosis (1). Food intake is an important factor that affects vascular reactivity. Both short-term feeding trials and acute experiments with test meals to assess atherogenic events taking place in the postprandial period have shown the potential of food for improving endothelial function, as isolated nutrients, such as n–3 PUFAs, L-arginine, tocopherol, phenolic antioxidants, folic acid, and magnesium; foods enriched with these nutrients; or healthy food patterns (12, 13, 69–72). Because sizeable amounts of all the nutrients listed above exist in walnuts, it is not surprising that both walnut diets and walnut meals were associated with improved endothelial function in 2 clinical studies (42, 45). In support of those observations, Davis et al (73) showed that walnut feeding reduced the expression of endothelin 1, a potent endothelial activator, in an animal model of accelerated atherosclerosis, and this effect could be attributed to the fat component of walnuts. Although no endothelial function studies have been performed after consumption of diets enriched with other nuts, they might be expected to also show beneficial effects because, with the exception of ALA, particular to walnuts, all nuts contain the bioactive nutrients described above.

CONCLUSIONS

Investigation of the molecular mechanisms underlying atherosclerosis and associated CVD showed that the disease has a complex cause beyond the accumulation of cholesterol on the arterial wall, with enhanced oxidative stress, a prominent inflammatory response, and abnormal vasomotor tone as interrelated and self-perpetuating phenomena. Novel circulating and functional biomarkers of these proatherogenic processes have been shown to be associated, independent of the lipid profile, with future cardiovascular events. Markers of oxidation, inflammation, and endothelial dysfunction are being intensively studied for important diagnostic and prognostic information

TABLE 2
Effects of nut consumption on novel biomarkers of cardiovascular risk and bioactive constituents of nuts that might explain them[1]

Effect on biomarker	Bioactive nut components
Reduced oxidation	Antioxidants
	α- and γ-Tocopherol
	Phenolic compounds
	Phytosterols
	Melatonin (walnuts)
	Selenium
	Monounsaturated fatty acids
Antiinflammatory effect	Antioxidants
	α- and γ-Tocopherol
	Phenolic compounds
	Ellagic acid (walnuts)
	α-Linolenic acid (walnuts)
	Magnesium
Improved endothelial function	L-arginine
	α-Linolenic acid (walnuts)
	Antioxidants
	α- and γ-Tocopherol
	Phenolic compounds
	Folic acid
	Magnesium

[1] Data on nut constituents and their presumed bioactivity were derived from the contents of a recent monograph on nuts and health outcomes (74).

directed to better CVD risk assessment and as potential treatment targets. Nut consumption has shown a consistent inverse association with incident CVD in prospective studies and a no less consistent cholesterol-lowering effect in feeding trials. As shown in this review, nuts also have the potential to favorably influence these novel CVD biomarkers, although evidence is still fragmentary, and additional studies are clearly warranted. As summarized in **Table 2**, many bioactive components of nuts, acting in isolation or most probably synergistically, might explain these beneficial effects. Although more work is needed to better understand the underlying biological mechanisms of cardiometabolic protection by these unique natural foods, it can undoubtedly be said that the whole, including the skin, is better than the parts. (Other articles in this supplement to the Journal include references 75–101.)

REFERENCES

1. Hansson GK. Inflammation, atherosclerosis, coronary artery disease. N Engl J Med 2005;352:1685–95.
2. Nedeljkovic ZS, Gokce N, Loscalzo J. Mechanisms of oxidative stress and vascular dysfunction. Postgrad Med J 2003;79:195–200.
3. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev 2006;86:515–81.
4. Vasan RS. Biomarkers of cardiovascular disease. Molecular basis and practical considerations. Circulation 2006;113:2335–62.
5. Fraley AE, Tsimikas S. Clinical applications of circulating oxidized low-density lipoprotein biomarkers in cardiovascular disease. Curr Opin Lipidol 2006;17:502–9.
6. Ferri N, Paoletti R, Corsini A. Biomarkers for atherosclerosis: pathophysiological role and pharmacological modulation. Curr Opin Lipidol 2006;17:495–501.
7. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction. Testing and clinical relevance. Circulation 2007;115:1285–95.
8. Keys A. Seven Countries: a multivariate analysis of death and coronary heart disease. Cambridge, MA: Harvard University Press, 1980.
9. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA 2002;288:2569–78.
10. Blomhoff R. Dietary antioxidants and cardiovascular disease. Curr Opin Lipidol 2005;16:47–54.
11. Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. Arterioscler Thromb Vasc Biol 2006;26:995–1001.
12. Brown AA, Hu F. Dietary modulation of endothelial function: implications for cardiovascular disease. Am J Clin Nutr 2001;73:673–86.
13. Kay CD, Kris-Etherton PM, West SG. Effects of antioxidant-rich foods on vascular reactivity: review of the clinical evidence. Curr Atheroscler Rep 2006;8:510–22.
14. Fung TT, McCullough ML, Newby PK, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2005;82:163–73.
15. Nettleton JA, Steffen LM, Mayer-Davis EJ, et al. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr 2006;83:1369–79.
16. Kelly JH Jr, Sabaté J. Nuts and coronary heart disease: an epidemiological perspective. Br J Nutr 2006;96(suppl 2):S61–7.
17. Mukuddem-Petersen J, Oosthuizen W, Jerling J. A systematic review of the effects of nuts on blood lipid profiles in humans. J Nutr 2005;135:2082–9.
18. Griel AE, Kris-Etherton PM. Tree nuts and the lipid profile: a review of clinical studies. Br J Nutr 2006;96(suppl 2):S68–78.
19. Ros E, Mataix J. Fatty acid composition of nuts. Implications for cardiovascular health. Br J Nutr 2006;96(suppl 2):S29–35.
20. Exler J, Weihrauch JL. Provisional table on the content of omega-3 fatty acids and other fat components in selected foods. Washington, DC: US Department of Agriculture, 1986. (Publication HNIS/PT-103.)
21. Vivancos M, Moreno JJ. beta-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. Free Radic Biol Med 2005;39:91–7.
22. Segura R, Javierre C, Lizarraga MA, Ros E. Other relevant components of nuts: phytosterols, folates and minerals. Br J Nutr 2006;96(suppl 2):S36–44.
23. Huynh NN, Chin-Dusting J. Amino acids, arginase and nitric oxide in vascular health. Clin Exp Pharmacol Physiol 2006;33:1–8.
24. Blomhoff R, Carlsen MH, Frost Andersen L, Jacobs DR Jr. Health benefits of nuts: potential role of antioxidants. Br J Nutr 2006;96(suppl 2):S52–60.
25. Wagner KH, Kamal-Eldin A, Elmadfa IU. Gamma-tocopherol an underestimated vitamin? Ann Nutr Metab 2004;48:169–88.
26. Seeram NP, Zhang Y, Henning SM, et al. Pistachio skin phenolics are destroyed by bleaching resulting in reduced antioxidative capacities. J Agric Food Chem 2006;54:7036–40.
27. Jiang R, Jacobs DR Jr, Mayer-Davis E, et al. Nut and seed consumption and inflammatory markers in the Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol 2006;163:222–31.
28. Mantzoros CS, Williams CJ, Manson JAE, Meigs JB, Hu FB. Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. Am J Clin Nutr 2006;84:328–35.
29. Salas-Salvadó J, Garcia-Arellano A, Estruch R, et al for the PREDIMED Investigators. Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. Eur J Clin Nutr 2008;62:651–9.
30. Reaven PD, Witzum JL. Oxidized low density lipoproteins in atherogenesis: role of dietary modification. Annu Rev Nutr 1996;16:51–71.
31. Berry EM, Eisenberg S, Friedlander Y, et al. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins–the Jerusalem Nutrition Study. II Monounsaturated fatty acids vs carbohydrates. Am J Clin Nutr 1992;56:394–403.
32. Jenkins DJ, Kendall CW, Marchie A, et al. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. Circulation 2002;106:1327–32.
33. Jenkins DJ, Kendall CW, Marchie A, et al. Almonds reduce biomarkers of lipid peroxidation in older hyperlipidemic subjects. J Nutr 2008;138:908–13.
34. Jenkins DJ, Kendall CW, Josse AR, et al. Almonds decrease postprandial glycemia, insulinemia, and oxidative damage in healthy individuals. J Nutr 2006;136:2987–92.
35. Hyson DA, Schneeman BO, Davis PA. Almonds and almond oil have similar effects on plasma lipids and LDL oxidation in healthy men and women. J Nutr 2002;132:703–7.
36. Durak I, Köksal I, Kaçmaz M, Büyükkoçak S, Çimen BMY, Öztürk HS. Hazelnut supplementation enhances plasma antioxidant potential and lowers plasma cholesterol levels. Clin Chim Acta 1999;284:113–5.
37. Kocyigit A, Koylu AA, Keles H. Effects of pistachio nuts consumption on plasma lipid profile and oxidative status in healthy volunteers. Nutr Metab Cardiovasc Dis 2006;16:202–9.
38. Garg ML, Blake RJ, Wills RBH, Clayton EH. Macadamia nut consumption modulates favourably risk factors for coronary artery disease in hypercholesterolemic subjects. Lipids 2007;42:583–7.
39. Hargrove RL, Etherton TD, Pearson TA, Harrison EH, Kris-Etherton PM. Low fat and high monounsaturated fat diets decrease human low density lipoprotein oxidative susceptibility in vitro. J Nutr 2001;131:1758–63.
40. Zambón D, Sabaté J, Muñoz S, et al. Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholesterolemic men and women: a randomized crossover trial. Ann Intern Med 2000;132:538–46.
41. Iwamoto M, Imaizumi K, Sato M, et al. Serum lipid profiles in Japanese women and men during consumption of walnuts. Eur J Clin Nutr 2002;56:629–37.
42. Ros E, Núñez I, Pérez-Heras A, et al. A walnut diet improves endothelial function in hypercholesterolemic subjects. Circulation 2004;109:1609–14.
43. Davis L, Stonehouse W, Loots du T, et al. The effects of high walnut and cashew nut diets on the antioxidant status of subjects with metabolic syndrome. Eur J Nutr 2007;46:155–64.

44. Fitó M, Guxens M, Corella D, et al., for the PREDIMED Study Investigators. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized, controlled trial. Arch Intern Med 2007;167:1195–203.

45. Cortés B, Núñez I, Cofán M, et al. Acute effects of high-fat meals enriched with walnuts or olive oil on postprandial endothelial function in healthy subjects and patients with hypercholesterolemia. J Am Coll Cardiol 2006;48:1666–71.

46. Mukuddem-Petersen J, Stonehouse W, Jerling JC, Hanekom SM, White Z. Effects of a high walnut and high cashew nut diet on selected markers of the metabolic syndrome: a controlled feeding trial. Br J Nutr 2007;97:1144–53.

47. Estruch R, Martínez-González MA, Corella D, et al., for the PREDIMED Study Investigators. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 2006;145:1–11.

48. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary α-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr 2004;134:2991–7.

49. Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM. Dietary α-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. Am J Clin Nutr 2007;85:385–91.

50. Reiter RJ, Manchester LC, Tan DX. Melatonin in walnuts: influence on levels of melatonin and total antioxidant capacity of blood. Nutrition 2005;21:920–4.

51. Rayman MP. The importance of selenium to human health. Lancet 2000;356:233–41.

52. Thomson CD, Chisholm A, McLachlan SK, Campbell JM. Brazil nuts: an effective way to improve selenium status. Am J Clin Nutr 2008;87:379–84.

53. Chen C-Y, Milbury PE, Lapsley K, Blumberg JB. Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. J Nutr 2005;135:1366–73.

54. Vivancos M, Moreno JJ. Effect of resveratrol, tyrosol and beta-sitosterol on oxidised low-density lipoprotein-stimulated oxidative stress, arachidonic acid release and prostaglandin E2 synthesis by RAW 264.7 macrophages. Br J Nutr 2008;99:1199–207.

55. Devaraj S, Leonard S, Traber MG, Jialal I. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. Free Radic Biol Med 2008;44:1203–8.

56. Halvorsen BL, Holte K, Myhrstad MC, et al. A systematic screening of total antioxidants in dietary plants. J Nutr 2002;132:461–71.

57. Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. Biochem Pharmacol 2006;72:1439–52.

58. Yu YM, Wang ZH, Liu CH, Chen CS. Ellagic acid inhibits IL-1 beta-induced cell adhesion molecule expression in human umbilical vein endothelial cells. Br J Nutr 2007;97:692–8.

59. Papoutsi Z, Kassi E, Chinou I, Halabalaki M, Skaltsounis LA, Moutsatsou P. Walnut extract (Juglans regia L.) and its component ellagic acid exhibit anti-inflammatory activity in human aorta endothelial cells and osteoblastic activity in the cell line KS483. Br J Nutr 2008;99:715–22.

60. Singh U, Jialal I. Anti-inflammatory effects of alpha-tocopherol. Ann N Y Acad Sci 2004;1031:195–203.

61. Calder PC. n–3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006;83(suppl):1505S–19S.

62. Zhao G, Etherton TD, Martin KR, et al. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. Biochem Biophys Res Commun 2005;336:909–17.

63. Paolisso G, Barbagallo M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. Am J Hypertens 1997;10:346–55.

64. King DE, Mainous AG III, Geesey ME, Woolson RF. Dietary magnesium and C-reactive protein levels. J Am Coll Nutr 2005;24:166–71.

65. Song Y, Ridker PM, Manson JE, Cook NR, Buring JE, Liu S. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care 2005;28:1438–44.

66. Song Y, Li TY, van Dam RM, Manson JE, Hu FB. Magnesium intake and plasma concentrations of markers of systemic inflammation and endothelial dysfunction in women. Am J Clin Nutr 2007;85:1068–74.

67. Jiang R, Manson JE, Stampfer MJ, Liu S, Willett WC, Hu FB. Nut and peanut butter consumption and risk of type 2 diabetes in women. JAMA 2002;288:2554–60.

68. Salas-Salvadó J, Fernández-Ballart J, Ros E, et al., for the PREDIMED Study Investigators. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. Arch Intern Med 2008;168:2449–58.

69. Sanderson P, Olthof M, Grimble RF, et al. Dietary lipids and vascular function: UK Food Standards Agency workshop report. Br J Nutr 2004;91:491–500.

70. Vita JA. Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr 2005;81(suppl):292S–7S.

71. Gornik HL, Creager MA. Arginine and endothelial and vascular health. J Nutr 2004;134(suppl):2880S–7S.

72. Shechter M, Sharir M, Labrador MJ, Forrester J, Silver B, Bairey Merz CN. Oral magnesium therapy improves endothelial function in patients with coronary artery disease. Circulation 2000;102:2353–8.

73. Davis P, Valacchi G, Pagnin E, et al. Walnuts reduce aortic ET-1 mRNA levels in hamsters fed a high-fat, atherogenic diet. J Nutr 2006;136:428–32.

74. Sabaté J, Salas-Salvadó J, Ros E, eds. Nuts: nutrition and health outcomes. Preface. Br J Nutr 2006;96(suppl 2):S1–2.

75. Rajaram S, Sabaté J. Preface. Am J Clin Nutr 2009;89(suppl):1541S–2S.

76. Jacobs DR Jr, Gross MD, Tapsell LC. Food synergy: an operational concept for understanding nutrition. Am J Clin Nutr 2009;89(suppl):1543S–8S.

77. Jacobs DR Jr, Haddad EH, Lanou AJ, Messina MJ. Food, plant food, and vegetarian diets in the US dietary guidelines: conclusions of an expert panel. Am J Clin Nutr 2009;89(suppl):1549S–52S.

78. Lampe JW. Interindividual differences in response to plant-based diets: implications for cancer risk. Am J Clin Nutr 2009;89(suppl):1553S–7S.

79. Simon JA, Chen Y-H, Bent S. The relation of α-linolenic acid to the risk of prostate cancer: a systematic review and meta-analysis. Am J Clin Nutr 2009;89(suppl):1558S–96S.

80. Pierce JP, Natarajan L, Caan BJ, et al. Dietary change and reduced breast cancer events among women without hot flashes after treatment of early-stage breast cancer: subgroup analysis of the Women's Healthy Eating and Living Study. Am J Clin Nutr 2009;89(suppl):1565S–71S.

81. Newby PK. Plant foods and plant-based diets: protective against childhood obesity? Am J Clin Nutr 2009;89(suppl):1572S–87S.

82. Barnard ND, Cohen J, Jenkins DJA, et al. A low-fat vegan diet and a conventional diabetes diet in the treatment of type 2 diabetes: a randomized, controlled, 74-wk clinical trial. Am J Clin Nutr 2009;89(suppl):1588S–96S.

83. Mangat I. Do vegetarians have to eat fish for optimal cardiovascular protection? Am J Clin Nutr 2009;89(suppl):1597S–601S.

84. Willis LM, Shukitt-Hale B, Joseph JA. Modulation of cognition and behavior in aged animals: role for antioxidant- and essential fatty acid–rich plant foods. Am J Clin Nutr 2009;89(suppl):1602S–6S.

85. Fraser GE. Vegetarian diets: what do we know of their effects on common chronic diseases? Am J Clin Nutr 2009;89(suppl):1607S–12S.

86. Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Cancer incidence in vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). Am J Clin Nutr 2009;89(suppl):1620S–6S.

87. Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). Am J Clin Nutr 2009;89(suppl):1613S–9S.

88. Craig WJ. Health effects of vegan diets. Am J Clin Nutr 2009;89(suppl):1627S–33S.

89. Weaver CM. Should dairy be recommended as part of a healthy vegetarian diet? Point. Am J Clin Nutr 2009;89(suppl):1634S–7S.

90. Lanou AJ. Should dairy be recommended as part of a healthy vegetarian diet? Counterpoint. Am J Clin Nutr 2009;89(suppl):1638S–42S.

91. Sabaté J, Ang Y. Nuts and health outcomes: new epidemiologic evidence. Am J Clin Nutr 2009;89(suppl):1643S–8S.

92. Rajaram S, Haddad EH, Mejia A, Sabaté J. Walnuts and fatty fish influence different serum lipid fractions in normal to mildly hyperlipidemic individuals: a randomized controlled study. Am J Clin Nutr 2009;89(suppl):1657S–63S.

93. Lampe JW. Is equol the key to the efficacy of soy foods? Am J Clin Nutr 2009;89(suppl):1664S–72S.
94. Badger TM, Gilchrist JM, Pivik RT, et al. The health implications of soy infant formula. Am J Clin Nutr 2009;89(suppl):1668S–72S.
95. Messina M, Wu AH. Perspectives on the soy–breast cancer relation. Am J Clin Nutr 2009;89(suppl):1673S–9S.
96. Lönnerdal B. Soybean ferritin: implications for iron status of vegetarians. Am J Clin Nutr 2009;89(suppl):1680S–5S.
97. Chan J, Jaceldo-Siegl K, Fraser GE. Serum 25-hydroxyvitamin D status of vegetarians, partial vegetarians, and nonvegetarians: the Adventist Health Study-2. Am J Clin Nutr 2009;89(suppl):1686S–92S.
98. Elmadfa I, Singer I. Vitamin B-12 and homocysteine status among vegetarians: a global perspective. Am J Clin Nutr 2009;89(suppl): 1693S–8S.
99. Marlow HJ, Hayes WK, Soret S, Carter RL, Schwab ER, Sabaté J. Diet and the environment: does what you eat matter? Am J Clin Nutr 2009; 89(suppl):1699S–703S.
100. Carlsson-Kanyama A, González AD. Potential contributions of food consumption patterns to climate change. Am J Clin Nutr 2009; 89(suppl):1704S–9S.
101. Eshel G, Martin PA. Geophysics and nutritional science: toward a novel, unified paradigm. Am J Clin Nutr 2009;89(suppl):1710S–6S.