

LSRO Report: The Scientific Evidence for a Beneficial Health Relationship Between Walnuts and Coronary Heart Disease

The Scientific Evidence for a Beneficial Health Relationship Between Walnuts and Coronary Heart Disease¹⁻³

Elaine B. Feldman, M.D.

Medical College of Georgia, Augusta, Georgia

ABSTRACT The author and four independent experts evaluated the intent and quality of scientific evidence for a potential beneficial health relationship between the intake of walnuts and the reduction and prevention of coronary heart disease. The report also addresses the supporting evidence for the health benefit of other tree nuts and selected legumes. Compared to most other nuts, which contain monounsaturated fatty acids, walnuts are unique because they are rich in n-6 (linoleate) and n-3 (linolenate) polyunsaturated fatty acids. Walnuts contain multiple health-beneficial components, such as having a low lysine:arginine ratio and high levels of arginine, folate, fiber, tannins, and polyphenols. Though walnuts are energy rich, clinical dietary intervention studies show that walnut consumption does not cause a net gain in body weight when eaten as a replacement food. Five controlled, peer-reviewed, human clinical walnut intervention trials, involving approximately 200 subjects representative of the 51% of the adult population in the United States at risk of coronary heart disease were reviewed. The intervention trials consistently demonstrated walnuts as part of a heart-healthy diet, lower blood cholesterol concentrations. None of these studies were of extended duration that would be essential for evaluation of the sustainability of the observed outcomes. These results were supported by several large prospective observational studies in humans, all demonstrating a dose response-related inverse association of the relative risk of coronary heart disease with the frequent daily consumption of small amounts of nuts, including walnuts. *J. Nutr.* 132: 1062S-1101S, 2002.

KEY WORDS • walnuts • coronary heart disease • nuts • legumes • polyunsaturated fatty acids

INTRODUCTION

This report focuses on the potential health benefit of walnuts in the reduction of the risk of coronary heart disease (CHD) and the prevention of heart disease. The goal was to review and analyze the scientific evidence on the effects of the intake of walnuts on risk factors for cardiovascular disease (CVD) and on the prevention of heart disease, including a consideration of the nutritional components in walnuts. As background, sections of this report summarize the relationship of CVD and blood lipids; the effects of fats and fatty acids on blood cholesterol; fatty acid effects on CVD risk factors other than blood lipids; and foods and nutrients other than fats found in nuts that may relate to the risk of heart disease.

Investigating the relation of diet to disease prevention can

encompass evaluating foods for specific nutrients or nonnutrient chemicals, alone or in combination; however, whole foods, or their constituents, may have disparate effects. An example is the relation of fruits and vegetables and their content of antioxidants to the prevention of heart disease or cancer. Because the detailed composition of individual meals consumed by humans is not well documented, attention to individual foods is necessary. Dietary studies rarely enroll sufficient subjects to evaluate the effect of a single change of diet on cardiovascular morbidity or mortality. More often investigators use surrogate endpoints or biomarkers, eg, blood lipids and lipoproteins or blood pressure (BP) that are risk factors for vascular disease. Other risk factors such as cigarette smoking, obesity, family history, glucose intolerance, level of physical activity, age, and gender may be confounders. Atherosclerosis is a progressive disease of the arteries; its pathogenesis continues to undergo active investigation.

BACKGROUND

Coronary heart disease and blood lipids

Research for more than 45 y has defined the relationship of CHD to total cholesterol (TC) and the major lipoprotein fractions. There is almost complete agreement that hypercholesterolemia [elevated TC and low-density lipoprotein-cholesterol (LDL-C)] is a risk factor for CHD. The ranges of con-

¹ Published as a supplement to *The Journal of Nutrition*. Guest Editor for this supplement publication was Elaine B. Feldman, Medical College of Georgia, Augusta, Georgia. This supplement is the responsibility of the guest editor to whom the Editor of *The Journal of Nutrition* has delegated supervision of both technical conformity to the published regulations of *The Journal of Nutrition* and general oversight of the scientific merit of each article. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, editor, or editorial board of *The Journal of Nutrition*.

² Prepared for California Walnut Commission, 1540 River Park Drive, Suite 203, Sacramento, CA.

³ For correspondence, contact Michael Falk, Life Sciences Research Office, 9650 Rockville Pike, Bethesda, Maryland 20814-3998. Tel: (301) 530-7030, fax (301) 571-1876; email: falkm@lsro.faseb.org

centrations that define the risk are agreed upon, and changes in risk of CHD in relation to changes in cholesterol concentrations have been demonstrated in prospective trials. Changes in the cholesterol concentrations of populations have been related to the incidence of CHD in numerous observational studies. The positive relationship of high-density lipoprotein-cholesterol (HDL-C) to the reduction of heart disease risk also has been defined. The contribution of genetic makeup to the lipid and lipoprotein risk factors is recognized conceptually. Any modulation of lifestyle may be limited by the inherent genetic makeup of the subject (see section on Fats and fatty acids in relation to blood lipids and lipoproteins and arterosclerosis).

CHD is the leading cause of death in the United States: 32% of females and 50% of males will develop CHD, and it is the cause of death in 24% of females and 31% of males. Increasing the serum TC level raises the risk of CHD and decreasing it will reduce risk. The risk of developing CHD is continuous over the range of serum TC levels, with moderate risk associated with levels exceeding 5.2 mmol/L (200 mg/dL), and high risk with >6.2 mmol/L (240 mg/dL). LDL-C levels can be classified similarly into low, moderate, and high risk. Coronary events are reduced by 2-3% for every 1% decrease in LDL-C, as reported in the Helsinki Heart Study (1). Small, dense LDL particles are associated with a tripling of the risk of myocardial infarction (MI) compared to the larger, more buoyant LDL particles. An elevated level of lipoprotein (a) [Lp (a)] also increases the risk of atherosclerosis. The smaller Lp (a) particles are more atherogenic, and may act by promoting LDL oxidation and decreasing endothelial-dependent vasodilatation and by enhancing retention of LDL in the arterial wall. Lp (a) may also have antifibrinolytic activity, an independent risk factor for atherothrombosis (2).

In contrast, HDL-C levels are inversely related to the risk of CVD. Risk is appreciably higher in subjects with HDL levels < 0.9 mmol/L (35 mg/dL), and risk decreases as HDL levels increase to > 1.6 mmol/L (60 mg/dL). The highest levels of HDL-C are considered a negative risk factor, ie, they reduce the risk of CVD. Coronary events are reduced by 3% for every 1% increase in HDL-C. The ratios of TC:HDL-C and LDL:HDL-C indicate varying degrees of the risk of CVD.

The results of a more recent multicenter randomized clinical study—the Veterans Affairs Cooperative Studies Program High-Density Lipoprotein Cholesterol Intervention Trial, as reported by Rubins and Robins (3) and Rubins et al. (4)—showed the benefit of raising HDL and lowering triacylglycerol levels, independent of effects on the LDL-C level. As a result of dietary intervention in this study, subjects showed no change in LDL-C, a 31% reduction in triacylglycerol, a 6% increase in HDL, a 22% reduction in CHD death and nonfatal MI, and a 29% decrease in stroke. The triacylglycerol level in blood is elevated by increases in energy, fat, carbohydrates, and alcohol. Recent data support the concept that higher triacylglycerol levels increase the risk of CHD, independent of HDL levels or other confounding factors of the dyslipidemic syndrome (ie, glucose intolerance, hyperinsulinism, obesity, and hypertension). This relationship applies especially to diabetic persons and females. Triacylglycerol levels > 2.6 mmol/L (100 mg/dL) increase the risk of CVD, independent of the usual accompanying low HDL. Fasting hypertriglyceridemia may be a stronger predictive risk factor than TC (5), as reported in a meta-analysis of 17 population-based studies on triacylglycerol levels and CVD. The Physicians' Health Study (6) also supports triacylglycerol as an independent risk factor of MI.

Other predictive risk factors for CVD may include levels of

fasting plasma insulin and apolipoprotein B (apo B), LDL particle size, and levels of circulating apoproteins. Absolute levels of apoproteins, or changes in lipoprotein particle size, or single amino acid mutations (as in apo-E isoforms) may be better predictors of CHD than lipid levels. It is not known whether these putative risk factors are responsive to dietary change or relevant beyond the specific subpopulations in which they have been evaluated, thus their value for planning dietary interventions is not clear. The relationship of apoprotein polymorphism to diet responsiveness is intriguing and under scrutiny. Elevated triacylglycerol levels may help identify individuals at high risk because of the associated predominance of small, dense LDL particles in these individuals. Elevated levels of insulin as a result of fasting are associated with impaired fibrinolysis and hypercoagulability in individuals with normal or abnormal glucose tolerance, thus enhancing the potential for acute thrombosis.

Although the relationship of the concentration and composition of blood lipids and lipoproteins to cardiovascular risk and atherosclerosis has been suspected for a century, the usefulness of various parameters in predicting disease or CHD events, or their responses to manipulations of lifestyle remain to be elucidated.

Fats and fatty acids in relation to blood lipids and lipoproteins and atherosclerosis

Current scientific consensus supports the value of using lifestyle modifications as a primary prevention mechanism to lower blood cholesterol in order to decrease heart disease risk. Not all of the population is responsive to such interventions, and CHD occurs in the presence of cholesterol levels within the "normal" range.

Feldman (7) has surveyed the association of diet and CVD. Dietary fats, especially saturated fat (SF) and cholesterol, raise the levels of serum/plasma TC, LDL-C, and triacylglycerol that increase CHD risk. Components of the diet may affect HDL-C, the lipoprotein that lessens CHD risk. Antioxidants (eg, vitamins E and C) may lessen the risk of CVD by decreasing oxidized LDL, which is more atherogenic. High blood levels of the amino acid homocysteine (Hcy) are associated with increased atherosclerosis and are decreased by the intake of folate and vitamins B₆ and B₁₂ (see section on Factors and mechanisms other than fats or blood lipids that affect CVD risk).

From the decline in cardiovascular mortality that was observed due to dietary restrictions during the Great Depression and World War II, we can infer the relation of the fat content and the fatty acid composition of the diet to the pathogenesis of atherosclerosis. The dietary fat-heart hypothesis was proposed by Keys (8) and Keys et al. (9) and related CHD rates to the intake of dietary fat, especially SF. A recent 25-year follow-up report (10) of the earlier study by Keys confirmed that the relative increase in CHD mortality due to a given cholesterol increase was similar in all cultures except Japan. A 0.52 mmol (20 mg) increase in serum TC corresponded to an increase in CHD mortality of 12%, increasing to 17% when adjusted for regression dilution bias.

Similarly, diets that are high in total fat (TF), SF, and cholesterol are atherogenic for many animal species. Cholesterol is found in the diet only in animal products and is not present in any plant sources. Long-chain saturated fatty acids (SFAs) in animal or vegetable fats raise plasma cholesterol levels and decrease LDL receptor activity. Paradoxically, these fats also raise HDL-C levels. Liquid vegetable oils with high concentrations of monounsaturated fatty acids (MUFAs) may

have a cholesterol-lowering effect and decrease LDL-C, but not HDL-C; some monounsaturated oils may lower triacylglycerol. Polyunsaturated fats (PUFAs) of the n-6 series in liquid vegetable oils decrease LDL-C; increased amounts of PUFA may lower HDL-C. The n-3 series of very long-chain, more unsaturated PUFAs found in fish and fish oils have variable effects on TC, LDL, and HDL-C, and lower the triacylglycerol levels. *Trans*-fatty acids are produced with some processes of partial hydrogenation of unsaturated liquid vegetable oils (in the United States, predominantly soybean oil). These fatty acids raise LDL-C somewhat less than the long- or medium-chain SFAs of butter, but, in contrast, lower HDL-C.

Theories of atherogenesis propose that LDL-C is the offending agent, delivering cholesterol to the arterial wall and initiating the pathogenic sequelae including endothelial injury. Endothelial injury initiates proliferation of vascular smooth muscle cells (SMCs) and conversion of monocytes to macrophages (cholesterol ester-laden foam cells) with proliferation of fibroblasts under the influence of growth factors and cytokines. Endothelial erosion is associated with atherosclerosis. Oxidized LDL accelerates the formation of foam cells, atheroma, and fibrous plaque. Plaque rupture initiates the events of MI. CHD progression is related directly to levels of TC and LDL-C, and inversely to HDL-C, especially HDL₂-C (a fraction of HDL-C) or the ratio of HDL₂-C:LDL-C. Levels of triacylglycerol > 6.5 mmol/L (250 mg/dL) increase the risk for MI and warrant intervention; more recent data on risk prevention suggest lowering this risk level, perhaps to 2.6 mmol/L (100 mg/dL). When LDL-C levels are reduced, cholesterol is removed from plaque thereby leading to regression of atherosclerosis. The National Cholesterol Education Program (11) has set a goal of LDL \leq 2.6 mmol/L (100 mg/dL) in secondary prevention of CHD (ie, in those who have already suffered an MI). This level of LDL usually parallels a TC value < 4.7 mmol/L (180 mg/dL).

Current theories of atherogenesis implicate plaque rupture with the release of a necrotic lipid core as the precipitating factor for thrombosis at the site and MI. Lipid-lowering, especially of LDL-C, stabilizes the plaque and reduces the risk of MI. Lower lipid levels may also decrease local concentrations of modified lipoproteins that have proinflammatory effects. With intervention, CVD mortality may be decreased by 25%, and incidence of MI by 50%. A significant drop in LDL-C has been shown to halt the progression of coronary atherosclerosis (12). The percent drop in LDL correlates 1:1 with the decrease in coronary events. Maximal dietary therapy typically reduced LDL-C levels by 0.39-0.65 mmol/L (15-25 mg/dL), or about 5-10%.

The 2001 National Cholesterol Education Program (13) proposed a multifaceted approach, designated "therapeutic lifestyle changes", to reduce the risk for CHD.

1. Lower intakes of SFA, including trans fatty acids (<7% of total calories) and cholesterol (less than 200 mg/day).
2. Maintaining total fat within the range of 25-35% of total calories provided SFA and trans fatty acids are kept low with higher intakes of MUFA (up to 20% of total calories) and PUFA (up to 10% of total calories).
3. Carbohydrate intakes (50-60% of total calories) should be derived from foods rich in complex carbohydrates including grains, especially whole grains, fruits, and vegetables. Recommended dietary fiber intake of 20-30 g/day. Dietary fiber has been estimated to lower LDL-C by from 3 to 10%. The American Heart Association (14) recommends a total dietary fiber intake of 25-30 g/day from food, about double the current intake in the United States.

4. Weight reduction for overweight or obese and sedentary to enhance LDL lowering, and modify other lipid and nonlipid risk factors.
5. Other therapeutic options for enhancing LDL lowering such as plant stanols/sterols (2 g/day) and increased viscous (soluble) fiber (10-25 g/day). The health benefit claim for oats (15) is based on a soluble fiber intake of 3 g/day (3 servings of oatmeal, 28 g each, each with 1 g of β -glucan soluble fiber). This soluble fiber can decrease TC and LDL-C by approximately 0.13 mmol/L (16).
6. Encouraging moderate physical activity to balance energy intake with energy expenditure and prevent or treat obesity, a condition that contributes to the atherogenic lipid pattern (17,18).

The American Heart Association recently issued a revised set of dietary guidelines (19) that are more individualized than before and that are food-based. There are four population-wide goals:

1. Achieve and maintain a healthy eating pattern that includes foods from all major food groups.
2. Achieve a healthy body weight (BW).
3. Achieve a desirable blood cholesterol and lipoprotein profile.
4. Achieve a desirable BP level.

Major guidelines are set for each goal. In the detailed American Heart Association scientific statement, nuts are specified in several guidelines. Nuts are referred to under Goal 1 in a paragraph about soluble fibers, viz., "Grains, vegetables, fruits, legumes, and nuts are good sources of fiber."

For Goal 3, nuts are listed under a guideline that limits foods with a high content of SF and cholesterol. The dietary recommendation is to "substitute grains and unsaturated fatty acids from fish, vegetables, legumes, and nuts." In a more detailed discussion, the report states that meta-analysis showed that 1 g of soluble fiber in the substituted foods (including nuts) would be expected to decrease LDL-C by an average of 0.057 mmol/L (2.2 mg/dL) (16). This section of the revised American Heart Association guidelines also discusses the evidence that foods rich in long-chain n-3 PUFAs confer cardioprotective effects beyond their effect on the improvement of the lipoprotein profile. These include reduction in sudden death (20,21), decreased risk of arrhythmia (22), lower plasma triacylglycerol levels (23), and a reduced blood-clotting tendency with eicosapentaenoic acid (20:5n-3, EPA) (24) and docosahexaenoic acid (22:6n-3, DHA) (25). In females, α -linolenic acid (18:3n-3) reduces risk of MI and fatal ischemic heart disease (IHD) (26,27). The report also cited the randomized controlled Lyon heart diet trial (28) that demonstrated the beneficial effects of α -linolenic acid on both coronary morbidity and mortality in patients with CHD. Nuts are specified as foods high in n-3 fatty acids, as are other plant sources (eg, flaxseed and flaxseed oil, canola oil, and soybean oil) and fatty fish. Under the heading "issues that merit further research," the section on n-3 fatty acid supplements states that intakes of EPA and DHA of approximately 900 mg/day could beneficially affect CHD mortality rates in patients with coronary disease.

Goal 4 recommends consuming a dietary pattern that emphasizes fruits, vegetables, LF dairy products, and is reduced in fat and cholesterol. The revised American Heart Association guidelines also discuss the study known as the Dietary Approaches to the Study of Hypertension (DASH) (29). The DASH clinical trial includes nuts in the dietary pattern and results in reduced systolic and diastolic BP, especially in African American hypertensive patients. Recent study results on

DASH have been published by Conlin et al. (30), Sacks et al. (31), and Svetkey et al. (32).

Factors and mechanisms other than fats or blood lipids that affect CVD risk

Other proposals have emphasized increasing the intake of n-3 fatty acids in fish and n-3 oils like flaxseed for prevention of CHD, in part because of their favorable action on prostaglandins. Cholesterol-lowering plant sterols, or their derivatives (ie, esters of sitosterol and sitostanol) have been added to fats and salad dressings. The FDA has authorized a health benefit claim for plant sterol/stanol esters and CHD (33). Other dietary components that lower cholesterol or reduce oxidized cholesterol include 25 g/day of soy protein (34), and perhaps 37 mg/day of the soy isoflavones (35), and the antioxidant vitamins E and C (explained below). The amount and type of dietary protein can affect levels of TC and LDL-C. Animal proteins are hypercholesterolemic, and plant proteins are hypocholesterolemic. This may be attributed to the content of the amino acids lysine and methionine in animal proteins and arginine in plant proteins (see section on Proteins in nuts as a factor in heart disease risk).

Moderate intake of alcoholic beverages (1–2/day, 10/week) raises HDL-C levels, and intake of rice bran or olive and canola oils may as well. The tocotrienols, plant sterols, or flavonoids in these oils may be partially responsible for an antiatherogenic effect. As vascular biologists derive more scientific data, they may find that foods influence atherogenesis by mechanisms that are less dependent on lipid:lipoprotein levels and more dependent on vascular reactivity and thrombus formation, in part mediated by nitric oxide (NO). Thus, the diet-heart hypothesis has evolved from the direct relationship of fat-to-heart-disease via blood lipids to more complex associations of foods, nutrients, and phytochemicals, along with genetic and molecular mechanisms of CVD, its pathology and clinical expression.

An elevated blood Hcy level is a risk factor for CVD. Hcy increases in relation to deficient intake or metabolism of folate, and vitamins B₆ and B₁₂. The following conclusions have been drawn from several recent studies on Hcy. First, Hcy is an independent risk factor for CHD equivalent in importance to hyperlipidemia and smoking, is a strong predictor of CVD mortality, and accounts for 10% of the attributed risk of CHD (36). Second, Hcy promotes prothrombotic changes in the vascular environment, arterial narrowing and endothelial cell toxicity, affects platelets and clotting control mechanisms, and stimulates smooth muscle cell proliferation. Third, investigators (37) have linked hyperhomocysteinemia with premature vascular occlusive diseases, viz., carotid occlusive disease, cerebrovascular disease, CHD, peripheral arterial occlusive disease, and veno-occlusive disease. The issue of Hcy and cardiovascular risk is addressed in a recent editorial (38) in the *American Journal of Clinical Nutrition* and in two articles written from opposite standpoints (39,40).

The relative risk (RR) of CVD is increased significantly when Hcy levels exceed ~15.8 $\mu\text{mol/L}$ (41). Apparently, there is a graded effect of the Hcy level on the risk of CVD (42). Elevated blood levels of Hcy can be normalized with vitamin supplements (0.2–1 mg folic acid, with or without 0.4 mg cyanocobalamin, 10 mg pyridoxal), potentially decreasing cardiovascular risk (1). Emphasis should be placed on meeting daily requirements for folate and vitamins B₆ and B₁₂ in the diet.

The possible role of antioxidant vitamins (ie, vitamins E and C) and dietary supplements in reducing CVD risk is under

investigation. The dose of vitamin E that may be effective and safe, and the minimum duration of treatment for protection, are unknown (43–45). The GISSI-Prevenzione trial in Italy (46), a multicenter, open-label design, with random allocation of 11,324 male and female patients to daily n-3 PUFA and/or vitamin E (300 mg) doses, reported that vitamin E administered alone had no statistically significant benefit on survival after MI. This conclusion was based on the combined endpoints and their individual components when analyzed according to the factorial design. However, a statistically significant beneficial effect of vitamin E was determined from the secondary analyses of cardiovascular deaths and the three component subsets, and was comparable to the beneficial effect of n-3 PUFA. The report noted that the vitamin E dose was in excess of any dose achievable through dietary intake, corresponding to 200 tablespoons (3.0 L) of olive oil daily, and is much higher than the recommended dietary allowances for optimum health in adults. Several prospective intervention studies have found no reduction in coronary disease with vitamin C supplementation (43,47–50).

Diets for primary and secondary prevention of coronary heart disease

Diets for primary or secondary prevention of CHD should:

1. be optimized for energy balance;
2. be optimized for fats, fatty acid concentrations and composition;
3. be primarily plant-based (since that improves the fat and protein composition) and include fruits and vegetables;
4. include soluble and nonsoluble fibers;
5. include oatmeal, oat bran, and psyllium;
6. include soy protein;
7. include food sources of folate and vitamins B₆ and B₁₂;
8. include antioxidants, (ie, vitamins, flavonoids, and trace minerals); and
9. be moderate in alcohol intake.

These nutrients can affect not only recognized risk factors such as lipids and lipoproteins, but also vascular mechanisms, clinical symptoms, and heart disease outcomes (such as morbidity and mortality).

DIET MODIFICATIONS AND HEART DISEASE RISK AND OUTCOMES

SFAs

It is well-established (51) that SFA increases the risk of heart disease by increasing the levels of TC, LDL-C, apoprotein B (apo B), and increasing the ratios of TC:HDL-C and LDL-C:HDL-C. The main sources of SFA in the diet are animal fats, particularly in dairy and meat products. Decreasing SFA to $\leq 10\%$ of energy will reduce TC and LDL-C.

MUFAs

MUFA sources shown to be effective in lowering risk of CVD include tree and ground (legume) nuts as well as popular liquid vegetable oils such as olive oil (“Mediterranean” diet) or peanut oil (“Asian” diet). Neither of these diets specifies a particular diet per se, but references the dietary habits of the inhabitants in the geographic regions where olive or peanut oils predominate and are preferentially used. MUFA-rich foods like avocado also should be acceptable. These additions provide more variety to the standard or “prudent” diet. These MUFA-enriched diets, however, are higher in fat and may be high in energy, thus presenting a potential problem to the

overweight. In contrast, substituting high-carbohydrate foods for fat also may add energy (and weight), especially if sugars are increased rather than whole grain complex carbohydrates.

n-6 PUFAs

Beneficial effects on the lipid profile of the intake of *n-6* PUFAs have been reported in many studies published since the late 1950s (52–64). This report is limited to recent reviews and meta-analyses of data from controlled studies. Seminal articles by Keys et al. (65) and Hegsted et al. (66) established predictive equations that related levels of fat intake to serum cholesterol levels. This established the initial prediction that PUFAs lowered cholesterol whereas SFAs increased cholesterol.

n-3 PUFAs

Salonen et al. (67), from Finland, studied the relationship of dietary intake of α -linolenic acid to BP. Intake obtained from 4-day food records (α -linolenic acid ranged from 0.6 to 4.1 g/day, mean 1.7 g/day) inversely related to mean resting BP. The authors did not provide data on the food sources of the fatty acid. The intake of α -linolenic acid ranged from 0.6 to 4.1 g/day, mean 1.7 g/day.

Another study, by Berry et al. (68), investigated the relationship of the fatty acid concentration in adipose tissue and BP in 399 free-living males in New York City. The investigators concluded that α -linolenic acid had a disproportionate association with BP, ie, a 1% increase in α -linolenic acid reduced BP 5 mm Hg. The authors cited the principal food sources of α linolenic acid in the diet as flaxseed (linseed) oil, legumes, and tree nuts (including walnuts and chestnuts).

Kang and Leaf (69) have reviewed the possible antiarrhythmic effect of *n-3* and *n-6* PUFAs, with a significant decrease in sudden death associated with their intake. The authors postulated that the fatty acids are incorporated into the sarcolemmal phospholipids of myocytes, perhaps binding to the protein of the sodium channel.

Mutanen and Freese (70) and Mutanen (71) reviewed the effects of PUFA on platelet aggregation, citing contradictory results. The evidence concerning linoleic acid (18:2 *n-6*) is not consistent, but intervention studies show increased platelet aggregation to agonists after high linoleate diets. Intake of α -linolenic acid either has no effect or leads to decreased platelet aggregation.

Reviews of the studies relating to the consumption of *n-3* fatty acids from fish oils have examined their possible role in preventing restenosis in patients after stent angioplasty. Results were variable, but none of these studies utilized α -linolenic acid. Instead, they used long-chain, more polyunsaturated marine oils, compared at times with olive oil. Effects on BP, and production of cytokines and growth factors, were investigated in addition to stenosis. Future studies with plant sources of α -linolenic acid would be interesting.

Effects of fats on lipid profile – meta-analyses

Results of a quantitative meta-analysis of 395 metabolic ward controlled studies of fat intake and blood cholesterol indicated that in the British diet, for example, replacing 60% of SFA by other fats and avoiding 60% of dietary cholesterol would reduce blood TC by 10–15% (about 0.8 mmol/L), with 80% of the reduction occurring in LDL-C (72). Median duration of studies was 1 mo. This dietary change could be accomplished by the isocaloric replacement of 10% of SFA

with complex carbohydrates, or the isocaloric replacement of 5% of complex carbohydrates by PUFA, and decreasing total daily dietary cholesterol by 200 mg. A 5% increase in MUFA had no significant effect on TC or LDL-C. A decrease in SFA provided the greatest cholesterol- and LDL-C-lowering effect; an increase in PUFA and decrease in cholesterol had about equal effect, about one quarter that of SFA reduction.

Mensink et al. (73), in a meta-analysis of 27 controlled diet trials, found that the most favorable lipoprotein risk profile for CHD was achieved if SFA were replaced by unsaturated fatty acid with no decrease in TF intake, assuming no increase in BW. Replacing 10% of total energy (TE) as SFA by carbohydrates would lower LDL-C by 0.34 mmol/L (13 mg/dL) and HDL-C by 0.12 mmol/L (4.67 mg/dL). Replacement by MUFA would reduce LDL-C by 0.39 mmol/L (15 mg/dL) and HDL-C by 0.31 mmol/L (1.2 mg/dL); replacement with PUFA would reduce LDL-C by 0.47 mmol/L (18 mg/dL) and HDL-C by 0.57 mmol/L (2.2 mg/dL).

Gardner and Kraemer (74) reported a meta-analysis to examine whether oils high in MUFA or PUFA had differential effects on serum lipid levels. The authors analyzed data from 14 small-to-medium diet trials and found no difference in the effect of increasing MUFA or PUFA on lowering TC or LDL-C, or in the effect on HDL-C. There may have been a slight TC-lowering effect of PUFA. They noted that other components in the oils and foods besides fatty acids may influence the outcome. MUFA studies included olive, canola, peanut, sunflower, and high-oleic safflower oils; PUFA studies included grapeseed, high-linoleic safflower oil, and corn oil. The authors considered the effects of decreasing or increasing each type of fatty acid by 10%. The SFA diet was 19% SFA, 13% MUFA, and 6% PUFA; the MUFA diet was 9% SFA, 19% MUFA, and 6% PUFA; and the PUFA diet was 9% SFA, 10% MUFA, and 16% PUFA. The authors compared *n-6* with *n-3* PUFA and *n-3* from plant sources with *n-3* from fish, suggesting that triacylglycerol-lowering may be attributed to a higher *n-3* content of diets. They indicated that associated micronutrients and factors other than fat in these oils or in foods could modify their effects on blood lipids. The authors reviewed the mixed findings that unsaturated fats may or may not have a differential influence on apoproteins, lipoprotein subclasses, blood glucose, and indexes of thrombosis and cancer. They suggested that outcomes other than serum cholesterol levels should be investigated as the basis for future dietary recommendations for the use of foods high in one unsaturated fat in preference to another.

INTERVENTION STUDIES WITH WALNUTS

The effect of the intake of walnuts on biomarkers of atherosclerotic CVD or on disease outcome was evaluated in five controlled, peer-reviewed, published studies in primarily healthy, normo- or hyperlipidemic human subjects (Studies 1–5) (see below). Another study (Study 6) mentions walnuts but provides no details on the amount ingested. Measurements were variously made on serum/plasma lipids and lipoproteins, plasma total fatty acid and lipid classes, and red blood cell (RBC) fatty acid. These studies have been carried out on small numbers of males and females of varying ages in the United States, Australia, New Zealand, Spain, and Israel.

Each study is summarized and reviewed from the perspective of the effects on biomarkers and indicators of heart disease (viz., lipids, lipoproteins, BP, pathology, and clinical events). The study is then examined for strengths and/or weaknesses in design, subject demography, dietary intervention, exposure dose, clinical and laboratory measurements, and data analysis.

Next the study is evaluated in terms of quality, consistency (ie, agreement) and magnitude of the effect, and the strength and relevance of the association of walnuts with the outcome. Finally, all of the studies are presented in chronological order of publication.

Study 1. Sabaté et al. (75) reported the effects of walnuts on serum lipid levels and BP. The investigators studied 18 healthy, normal weight, young (mean 30 y) adult males with “normal” lipid levels (20th–80th percentile) and BP, who did not eat nuts frequently. The subjects were placed on a National Cholesterol Education Program/American Heart Association Step 1 diet (30% fat), either with or without 20% of energy from walnuts, in a single-blind, randomized, crossover design with two 4-week feeding periods. Walnuts reduced the TC, LDL-C, and HDL-C values by 12% (22 mg), 16% (18 mg), and 5% (2 mg), respectively; all changes were statistically significant. There was no effect on BP. Triacylglycerol levels tended to decrease, but not significantly; the ratios of TC:HDL-C and LDL-C:HDL-C decreased significantly. The Walnut diet caused a slight increase in the intake of TF. SFA intake decreased by one-third, PUFA increased 80%, and cholesterol declined 50% in comparison to the Step 1 reference diet (RD), which included no walnuts. Comparing the Walnut and RD periods, the investigators noticed that the fatty acid composition of serum cholesterol esters reflected significant decreases in oleic (18:1 n-9) and arachidonic (20:4 n-6) acids and increases in linoleic and α -linolenic acids in the Walnut diet.

Although there was a 5-day run-in period, there was no washout between the 4-week duration of each diet (with or without walnuts). The study personnel were blinded to the patients’ study sequence, ie, one group was consuming the Walnut diet first and then crossed over; the other group was eating the diet without walnuts first and then crossed over. The nutrition research kitchen provided all meals; common, normal food items comprised the menus. The Walnut diet substituted three servings (28 g each) of walnuts daily (for a total of 84 g daily per 2,500 kcal) in place of other foods. Walnuts were used for snacks, mixed in salads and breakfast cereals, or cooked with dinner entrees. The composition of duplicate samples of the two study diets was determined by analyses of samples collected randomly. There were no side effects from the Walnut diet.

Lipid and BP measurements were standardized and validated. The two-tailed *t* test was used for statistical analyses.

The major difference between the two diets was the increase in PUFA in the Walnut diet, with the percent of α -linolenic acid tripling and total PUFA increasing 2.5-times. The response of the subjects was consistent regardless of the sequence of the diets, baseline lipid values, or body size. During the Walnut diet, walnuts contributed 55%, 14%, and 10% of TF, protein, and fiber, respectively.

The investigators concluded that replacing a portion of the fat in a cholesterol-lowering diet with walnuts further lowers serum cholesterol levels and produces a more favorable lipoprotein profile. They suggested that an increase in walnut consumption from a current average of 4 g/week to 28 g/day would lower cholesterol and LDL-C by 4 and 6%, respectively. They also suggested that incorporating walnuts into the diet as snacks, or components of desserts, breads, or entrees would be acceptable to most people as part of a cholesterol-lowering diet.

The investigators noted that their study did not include females or subjects with hypercholesterolemia, and included only younger subjects, and that 4 weeks was a short interval. The results, therefore, could not be extrapolated directly to the

population at higher risk of CHD. The issue of free-living subjects was not addressed.

This study was well-designed and controlled, insofar as a diet study with a whole food cannot be blinded to the subject. The small number of subjects, consisted only of young adult males; nevertheless, the dietary intervention was practical for the “real world” and a healthy population. A 4-week study is enough time in which to detect changes in blood lipids, but not long enough to address maintenance. The energy intake was controlled, but the proportion of fat energy from walnuts was too high to be practical. The extrapolation to one-third of that intake (ie, 28 g) and prediction of appreciable lipid-lowering need to be validated by direct study at that level. The magnitude of the lipid effect (cholesterol and LDL-C reduction) is significant and could provide risk reduction for heart disease for the population that is comparable to oatmeal and oat bran (15,76) or soy protein (77,78). The 5% fall in HDL is noteworthy, but is counterbalanced by the significant decrease in the ratios of cholesterol and LDL:HDL. The trend to decrease TGs by 10% might achieve statistical significance with a small increase in the number of subjects (perhaps to 25 individuals).

Study 2. Abbey et al. (79) studied 16 normolipidemic males, mean age of 41 y, with a 36% fat diet for periods of 3 weeks. The investigators compared a diet enriched with peanuts and coconut that was similar to the usual Australian diet in fat composition (SFA:MUFA:PUFA ratios) with an almond-enriched diet and one enriched in walnuts (68 g/day, 46 g fat). The nuts provided about half of the fat energy. All subjects were fed the diets in the same order. The Walnut diet was continued for an additional 3 weeks. Both almonds and walnuts similarly lowered TC and LDL-C significantly, ~6 and 10%, respectively, after 3 weeks compared with the RD. The HDL-C and triacylglycerol values did not differ among the three diets. The plasma fatty acid composition with the Walnut diet showed significant decreases in oleic and arachidonic acids and significant increases in linoleic and α -linolenic acids. Plasma and dietary fatty acid composition were measured as were plasma lipids and lipoproteins using validated methods. Data were analyzed by paired *t* tests.

The dietary intake was monitored by food records, with special emphasis on fat intake, and reviewed by the dietitian. The diets were well matched. The investigators were concerned about possible adverse effects of the high PUFA content of the Walnut diet on lipid peroxidation and the formation of oxidized LDL, although this was not studied. The relatively high content of tocopherols in walnuts might be protective (Table 1). They also discussed the possible influence on the results of elevated myristic acid (14:0) in the RD. They suggest that PUFA- and MUFA-rich nuts should be included in the diet as a replacement for some of the SFA.

The study design was reasonable and controlled, although the authors did not state whether any investigators or staff were blinded to the intervention. The number of subjects was small and was composed only of males; nevertheless, the intervention was practical for the “real world” and a healthy population. A 3-week study is minimally adequate to detect changes in blood lipids, but the Walnut group ingested this diet for 6 weeks, still a relatively short time that cannot address long-term effects. The energy intake was controlled at a higher level, ie, 36% of TE, representing more closely the usual fat intake of the population. This level may be practical for the “real world” as it requires only substitution of fat, rather than its decrease. The proportion of fat energy from walnuts was higher than is practical. An extrapolation to one-half that intake (ie, 34 g) could yield effects similar to Sabaté’s (75)

prediction of appreciable lipid-lowering, and needs to be validated by a clinical study at that dose. The magnitude of the lipid effect (cholesterol and LDL-C reduction) is significant and could provide risk reduction for heart disease for the population that is comparable to oatmeal and oat bran (15) or soy protein (78). The lack of effect on HDL is encouraging and may reflect either the lower level of intake of walnuts or the longer duration of feeding. It is interesting that the results with almonds did not differ from those achieved with walnuts, despite the difference in fatty acid composition, ie, almonds are high in MUFAs and walnuts in PUFAs (Table 1).

Study 3. Chisholm et al. (80) reported a study of 21 males (16 with full dietary records), mean age 45 y, with moderate hypercholesterolemia (polygenic hyperlipidemia), without CHD. The randomized, crossover design compared the effects on lipid metabolism for 4 weeks of two "LF" diets (ie, 30 and 38% TE). A diet with 38% energy from fat is not LF, as described by the investigators. Walnuts (78 g/day) were included only in the 38% LF diet. With the Walnut diet, the significant decreases in TC (4%) and LDL-C (8%) compared to the baseline diet, were about twice that observed with the 30% LF diet. HDL-C increased significantly (42%) with the Walnut diet compared to baseline, and apo B levels decreased significantly (10%) with the Walnut diet compared to baseline, with no significant change from baseline with the 30% LF diet. Neither triacylglycerol nor apo-A levels differed from baseline with either the Walnut or 30% LF diet. Comparing the Walnut diet and RD, the investigators found that the fatty acid composition of plasma triacylglycerol, cholesterol esters, and phospholipids showed significant decreases in 18:1 and 20:4 and increases in 18:2 in the three lipid classes, with an increase in 18:3 in triacylglycerol. Investigators ascribed the absence of more extensive differences between the two diets as possibly being caused by the relatively low levels of myristic acid in both. They noted that the Walnut diet had significantly less SFA, more PUFA, and less cholesterol than the 30% LF diet and recommended that an increase in TF be avoided when nuts are included in the diet.

The experimental design included a run-in period on a LF diet for 1 week before randomization into two groups, with one group continuing on the 30% LF diet, and the other switched to the Walnut diet. There was no washout between crossover periods. Dietary intake was recorded on 2 days of each week of the study. Meals were eaten at home, with individualized menus and recipes; walnuts were provided. Walnuts contributed 20% of the TE of the diet. The lipid measurements were validated for precision and accuracy, and the variances were provided. Data were analyzed using ANOVA with repeated measurements.

Despite the unintended 8% increase in TE from fat on the Walnut diet, the two diets were isocaloric. The subjects did not differ in BW or weight gain with either one of the diets. The investigators concluded that walnuts influence the plasma fatty acid profile in a way expected to reduce the risk of CHD, and is thereby cardioprotective. They were unable to demonstrate convincingly that the Walnut diet was superior to the 30% LF diet in improving the lipoprotein profile. They cautioned that an increase in TF should be avoided in order to improve study design in future interventions with nuts or in advising an increase in nut consumption.

This study did not achieve the desired fat content for the Walnut diet that would provide a better comparison with a LF diet. There was a modest lowering of cholesterol and LDL-C with both diets, especially considering the high intake of walnuts. Again, the number of subjects was small and consisted only of males. These subjects, however, were hyperlip-

idemic, rather than representative of the healthy population. This 4-week study was adequate to detect changes in blood lipids, but inadequate in terms of addressing sustainability over time. The energy intake was controlled, but the proportion of fat energy from walnuts was too high to be practical. The extrapolation to one-third of that intake (ie, 26 g) would not produce significant enough reduction of cholesterol and LDL-C risk factors for heart disease (ie, 1–2%) to indicate significant protection. The notable increase in HDL-C levels could provide a reduction in risk for heart disease for this population.

Study 4. Zambón et al. (81) reported on the Barcelona Walnut trial. The randomized, crossover design feeding study was carried out on 23 females and 26 males with polygenic hypercholesterolemia, mean age 56 y. Patients were recruited from a lipid clinic where they had been placed on a cholesterol-lowering "Mediterranean" diet. They were free of disease other than CHD (7 subjects), took no lipid-lowering medications or dietary supplements, and did not consume nuts frequently. A high-MUFA diet ("Mediterranean" diet consisting of olive oil and natural food stuffs) was compared to a high-PUFA diet (walnuts, 56 g/day) during 6-week feeding periods with random assignment to one of two sequences. There was a 1-week pretrial period for indoctrination and counseling, and no washout period between diets. The MUFA diet was 30% fat, 5% SFA, 21% MUFA, and 4% PUFA, and the PUFA diet was 33% fat, 5% SFA, 16% MUFA, and 12% PUFA. Raw, shelled walnuts were provided daily in packages varying between 41–56 g (8–11 walnuts), affording 18% of TE. Walnuts were consumed in desserts or salads, or as snacks. Diets were monitored by unannounced weekly telephone 24-h recalls. Serum lipids, Lp (a), apoproteins A-1 and B, and LDL resistance in vitro to oxidative stress were measured, as well as the fatty acid composition of LDL lipids in blood samples obtained at 5 and 6 weeks of each dietary period.

TC decreased by 9% on the Walnut diet (by 5% on the control diet); LDL-C declined by 11% (by 6% on the control diet), with parallel changes in apo B levels. Lp (a) levels were decreased (9 vs. 3%) with the control diet. The change in Lp (a) levels was statistically significant only in males and in subjects with levels of ≥ 0.78 mmol/L (30 mg/dL). There were no significant differences in the effects of the two diets on levels of HDL-C, triacylglycerol, or apo A-1 (both diets decreased these variables). The LDL:HDL ratio declined 8% with the Walnut diet and was unchanged with the control diet. Resistance of LDL to oxidative stress was preserved during the Walnut diet. There was no difference between the Walnut and control diets in the α -tocopherol content or in the lag time of conjugated dienes (CDs) formation during copper-induced oxidation. Oleic acid was decreased, whereas linoleic and α -linolenic acids were increased in LDL-cholesterol ester fatty acids, LDL phospholipid, and triacylglycerol with the Walnut. There was no evidence of a carryover effect.

BW was stable and most subjects tolerated the Walnut diet well, although a few reported mild symptoms of postprandial bloating or heaviness. The energy value and nutrient composition of the two diets (other than the fat) had minor differences besides lower cholesterol content in the Walnut diet, and were insufficient to explain the differences in blood lipids and lipoproteins that were observed with the diets.

Investigators concluded that substituting walnuts for part of the MUFA in a cholesterol-lowering "Mediterranean" diet further reduces TC and LDL-C in males and females with hypercholesterolemia. The Walnut diet effect was observed without changing dietary SFA. They suggested that nonfat components of the walnut matrix may influence blood lipids, and that the lipid effects of whole walnuts should be compared

with that of walnut oil. They also noted that the content of α -linolenic acid in walnuts may reduce the risk for CHD deaths through its antiarrhythmic properties or other anti-atherogenic effects (see below). They proposed that even greater benefits than they encountered studying the "Mediterranean" diet would be obtained by replacing the traditional "Western" dietary fat with walnuts.

Although the results show no change in triacylglycerol levels with either diet, an asterisk in Figure 2 in Zamboni (81) indicates a significant lowering of triacylglycerol with the Walnut diet. This would sustain evidence that walnuts have another favorable lipid effect in addition to those on cholesterol and lipoproteins. This study was well designed and controlled. The number of subjects was greater, included females and males, with a broader age range, and with elevated lipid levels. The 6-week study is not considered long-term. The caloric intake was controlled, but the TE from fat was higher with the Walnut diet, achieving a moderate fat rather than a LF intake. The proportion of fat calories from walnuts was double that considered practical. The extrapolation to half of that intake (ie, 25 g) and prediction of 4-5% cholesterol or LDL cholesterol-lowering needs to be validated by direct clinical study at that intake level. The magnitude of the lipid effect could provide risk reduction for heart disease for the population. The 3% fall in the HDL level is counterbalanced by the significant (8%) decrease in the ratio of LDL:HDL. A significant decrease in triacylglycerol would be an added benefit. The absence of an increase in oxidative stress with the Walnut diet also is favorable. The strength of this intervention diet is that the SFA content was the same for the two diets, so that the increase in PUFA was the significant change and was not accompanied by a decrease in an "unfavorable" nutrient.

Study 5. Almario et al. (82) reported the effects of a Walnut diet on plasma lipids and lipoproteins in patients with combined hyperlipidemia. Four diets were studied: the habitual diet (HD) (31% fat) and a LF (20% fat) diet; and the same diets supplemented with walnuts (48 g/8460 kJ). Thirteen postmenopausal females and 5 males, age 60 ± 8 y, completed the four periods. Comparing the Walnut diet with the HD and LF diets, patients lost weight on the LF diet, but did not gain weight with either Walnut diet, despite an increased TE intake of 20 and 23%, respectively.

During the LF + walnuts period, decreases in TC (8%) and LDL-C (12%) were significant, compared with the LF diet alone. Triacylglycerol did not differ among the four diets, and cholesterol was decreased significantly in intermediate density lipoprotein (IDL) and redistributed from the more atherogenic small, dense LDL to larger LDL particles. Plasma apo B was not effected by walnut supplementation but decreased with the LF compared to the HD. The HDL-C decreased (10%) significantly when comparing either the walnut supplemented HD or LF to HD. Apo A-1 increased with the addition of walnuts to the HD. Apo A-1 decreased during the LF diet and remained low after the addition of walnuts. Adding walnuts to the HD or the LF diet alone caused significant shifts in cholesterol that redistributed it from larger into smaller HDL particles. Lipid particle changes occurred in the absence of lipid lowering, suggesting a favorable antiatherogenic effect of walnuts that is independent of any changes in circulating lipid levels.

The patients' health was stable and their lifestyle was monitored. All patients followed the diet according to the following sequence: 4 weeks on the HD diet then 6 weeks each on the HD + walnuts, LF diet, LF + walnuts, respectively. There were no washout periods. Patients were counseled individually before the LF diet intervention and with group sessions during those periods.

The quantity of walnuts was selected to provide an amount of α -linolenic acid similar to the amount that lowered triacylglycerol in fish oil studies. Walnuts were consumed mainly from prepacked rations that were provided. Although the patients were seen and blood samples were drawn at mid- and endpoints of each period, only the terminal samples were used for data analysis. Lipoprotein particle sizes were determined by nuclear magnetic resonance techniques. Diets were monitored from 7-day food records for each period. Data analysis used general linear modeling procedures and correlations.

The walnuts added to the HD increased the fat content from 31 to 37%. Linoleic acid intake increased from 11 to 30 g and α -linolenic acid from 1.3 to 5.4 g. The SFA in HD and HD + Walnuts were 11 and 10%, respectively. MUFA were 12 and 13% and PUFA were 6 and 16% in HD and HD + walnuts, respectively. During the LF diet, the TE decreased by about 12% compared to the HD; this was reversed during the LF + walnuts diet. The LF diet contained 20% fat, 8% SFA, 8% MUFA, and 5% PUFA. LF + walnuts contained 34% TF, 8% SFA, 12% MUFA, and 16% PUFA. Addition of walnuts to either the HD or LF diet increased plasma concentrations of linoleic and α -linolenic acids and decreased palmitic (16:0), oleic, and arachidonic acids without changing concentrations of EPA and DHA.

The investigators suggest that the absence of effect of the LF diet is because these hyperlipidemic subjects were already following a diet restricted in fat and SF. They noted that the cholesterol-lowering caused by adding walnuts to the LF diet could not be explained in terms of decreasing SFA, but rather was a specific effect of the PUFA and MUFA content. They suggest further examination of possible effects of PUFA on lipoprotein metabolism via effects on lipoprotein lipase, hepatic lipase, lecithin-cholesterol acyltransferase, or cholesterol ester transfer protein, which are all involved in lipoprotein metabolism. Walnuts have a unique elevation of both n-6 (linoleic) and n-3 (α -linolenic) acids compared to other nuts. Their metabolic effects should be specifically elucidated and not extrapolated from other unsaturated fats and oils, even of similar fatty acid composition (eg, soybean and wheat germ oils).

This study was well designed and controlled. A small number of hyperlipidemic females and males were studied and fed diets for 6 weeks. Adding walnuts to either diet caused variation in the caloric intake and increased TE by $\geq 20\%$. This might be expected when adding walnuts to a LF diet but not, perhaps, to the HD. Also, the LF diet provided only 82% of the TE of the HD. The walnut intake is about twice that which is practical to include in the diet. The extrapolation to half that intake (24g) and prediction of appreciable lipid-lowering needs to be validated by direct study at that level. The magnitude of the lipid effect (cholesterol and LDL-C reduction) is significant and could reduce the population's risk for heart disease to an extent that is comparable to oatmeal and oat bran (15) or soy protein (78). The fall in HDL is noteworthy, although the LF diet produced a similar decrease. The ratios of cholesterol and LDL:HDL were not calculated. The intriguing findings of changes in lipoprotein particle sizes and composition need confirmation. The results obtained by the investigators from the emerging nuclear magnetic resonance methodology need to be related to the traditional procedures.

Study 6. In the Jerusalem Nutrition Study (83), walnuts were one component of a high-PUFA diet study, although there were no data on the amount of walnuts subjects consumed. The intervention study compared 32% fat, high-MUFA and -PUFA diets in 26 healthy, normolipidemic,

young males who were randomized to either diet in a 12-week crossover design. The MUFA diet was 8% SFA, 16% MUFA, and 7% PUFA with fat sources from olive oil, avocado, and almonds. The PUFA diet was 7% SFA, 6% MUFA, and 16% PUFA with fat from safflower and soy oils and walnuts. There was a 4-week washout before cross over to the other diet for an additional 12 weeks.

The PUFA diet reduced TC and LDL-C by 16%, whereas the MUFA diet reduction was 10%. There was no change in HDL-C or triacylglycerol. There was an increase in response to oxidative stress as determined by measurements of thiobarbituric acid reactive substances (TBARS) with the PUFA vs. MUFA diets. LDL receptor activity and lipoprotein profiles and composition (studied in about half the subjects chosen at random) were not significantly different between the two diets.

All food, consisting of natural and common food items, was served in a central kitchen. There was a 4-week run-in period on their usual diet (41% fat, 10% SFA, 12% MUFA, and 16% PUFA). Menus were prepared in 12-day rotations. Samples of the diet were analyzed for fatty acid composition by gas-liquid chromatography. Standard procedures were used to measure the lipids and lipoproteins. Statistical analysis was by means of the *t* test, comparing the means of two determinations at 10 and 12 weeks of the diets and two baseline samples. Dietary compliance by the subjects was monitored by determining the fatty acid composition of the lipids in the RBC membrane. The investigators demonstrated a seasonal effect on fatty acid levels, especially of triacylglycerol, but also of LDL-C, that might be related to dietary changes in Israel vis-à-vis religious observances.

This study focused on the demonstration that MUFAs were equivalent to PUFAs in cholesterol-lowering. In fact, they showed a better effect by PUFAs, which included walnuts as a source. The amount of walnuts was not specified, however, which makes this study interesting but probably not suitable for inclusion in this report as a walnut intervention study. Rather, it supports the direct intervention data. The study resembles, in part, the comparison in Abbey's study (79) of an Almond diet with a Walnut one. The study shows that PUFAs do not adversely affect HDL, but also supports an adverse effect of PUFAs on oxidative stress (see section on Possible adverse effects of walnut components).

Summary of clinical human intervention trials with walnuts

Summaries of the six clinical human intervention studies published or in press are presented in **Table 2**. They are consistent in showing decreases in TC and LDL-C that should lower risk of CHD. These results are achieved with intakes of walnuts that would amount to two or three servings daily. There appears to be a null effect on triacylglycerol. Effects on HDL-C are inconsistent, with some studies showing a decrease, others no change, and one an increase. Not all the studies have included the ratios of TC:HDL-C or LDL-C:HDL-C. Where evaluated, these ratios decreased, indicating lessening of risk, even when HDL-C is decreased. In one trial, although HDL-C decreased, apo A-1 increased, providing another favorable outcome. A few studies have included apo B and have shown decreases as well, again favoring risk reduction. These results have been achieved whether the walnuts were included in a usual diet higher in fat, a Step 1 diet, a LF diet, or in a diet already increased in PUFAs.

Although the number of subjects included in these six trials is small, they are representative of the 51% of the adult

population in the United States now at higher risk of CHD (33). Among the deficiencies in these trials is that no study included a large number of subjects, encompassing males and females, of a broad age range, and studied over a long period of time. Each involved a level of walnut consumption that is not considered practical in the "real world." No significant adverse effects have been reported in any of the studies. Although the walnut consumption may add calories, net BW gains did not occur.

The concern about increased oxidative stress in these studies mirrors the concern with diets that increase PUFAs. The data are inconsistent; the one Walnut trial that examined this possibility showed no adverse effect. The effect of walnut ingestion at a practical level, ie, one serving daily, has not been evaluated. The walnut effect at higher intakes (two to three servings daily) resembles that of oat fiber and soy protein, where the amounts tested in trials exceed usual intakes.

The mechanism of the walnut effect is unclear. The diets used generally reflect a ratio of SFA:PUFA of 1:2, which was a dietary recommendation made early in efforts to reduce the risk of heart disease. Investigators have claimed that the risk reduction with walnuts may exceed that predicted from their fatty acid concentrations and composition, suggesting other factors (see below). Walnuts are unique among nuts in that both α -linolenic acid and linoleic acid levels are increased. The dietary intake of walnuts significantly increased levels of both PUFAs in the body, thus demonstrating bioavailability. Risk factors of interest that were addressed only in a single trial include Lp (a), which has not been shown to respond readily to dietary intervention, and LDL and HDL particle sizes, which are emerging as more specific risk factors than the levels of LDL or HDL-C. These particle sizes may have different effects on risk (ie, not all LDLs are "bad" and not all HDLs are "good").

A possible effect on Hcy also needs to be examined in a strong study. Similarly, the significance of the lysine:arginine ratio remains to be defined.

OBSERVATIONAL STUDIES ON CONSUMPTION OF WALNUTS AND OTHER NUTS AND CORONARY HEART DISEASE

Large-scale observational studies permit evaluation of the effects of nut consumption on CVD risk, mortality, and morbidity. Such studies published in the last decade include subject demography, cross-sectional, and case-control design, and have demonstrated a favorable effect of nut consumption and, specifically, walnut consumption, on reducing the risk of atherosclerotic CVD morbidity and mortality by 30–50%. The frequency and quantity of nut consumption by vegetarians, and as part of plant-based "Mediterranean" or "Asian" diets, are associated with cardiovascular risk reduction and are inversely related to all-cause mortality. The effects are similar in males, females, the elderly, Caucasians, and African Americans, and have been shown in four large prospective cohort studies (viz., the Adventist Health Study [AHS], the Nurses' Health Study [NHS], the Iowa Women's Health Study, and the Physician's Health Study). Summaries of these observational studies are presented in **Table 3**.

Nuts as a group

The AHS is a large, prospective study of Seventh-day Adventists (SDAs) in California (84–87), in which 31,208 non-Hispanic white subjects were followed for 6 y (1977–1982). Excluding people with heart disease or diabetes, at

baseline 26,473 males and females over age 25 were evaluated for first events of MI or CHD. Nut consumption ranged from never to daily. Nut consumption was related inversely to nonfatal MI or death from IHD, or to all-cause mortality. RR of MI and IHD in persons who ate nuts more than 4 times/week were half the RR of subjects who consumed nuts less than once weekly (RR 1). People who ate nuts 1–4 times/week had a 22% reduced risk of acute MI, compared with those eating nuts less than once a week.

The population was predominantly nonsmoking, did not use alcohol, and generally followed a lacto-ovo-vegetarian diet. Diet was assessed by a semiquantitative food frequency questionnaire (FFQ). Participants' health status was queried annually, and medical records were reviewed for the diagnosis of CVD, nonfatal or fatal, according to the International Classification of Diseases codes. The association of nuts and CVD was undiminished when analyzed in the elderly subgroup, and did not differ by sex, BP, relative weight, nor was there any significant confounding by other foods. Although the investigators did not distinguish between different kinds of nuts, a substudy showed that 32% of nuts consumed were peanuts (legumes or ground nuts), 29% were almonds, 16% were walnuts, and 23% were other kinds of nuts. The authors concluded that frequent consumption of nuts might be protective for both fatal and nonfatal CHD events.

Fraser (85) has proposed that high nut consumption postpones the development of IHD for several years and confers an 18% lifetime risk reduction of CHD compared with 30% in consumers of low quantities of nuts.

There are several publications by investigators involved in the AHS prospective observational studies on SDA cohort populations. Fraser and colleagues (88) estimated the effects of particular practices or risk factors, such as consumption of nuts, on the lifetime risk of CHD and the time of first expression in 26,321 non-Hispanic, white SDAs. Their analyses:

- demonstrated that the age of onset of CHD in both males and females was delayed by about 4 y when comparing high (5×/week) to low (rare) nut consumption
- predicted that life expectancy free of CHD was significantly longer (5.6 y, $p > 0.05$) with high nut consumption
- predicted lifetime risk of CHD was 12% less in high nut consumers compared to low ($p < 0.001$)
- estimated that the lifetime risk of CHD for competing causes was 0.77 ($p < 0.001$) for high nut consumption.

A population of African American SDAs in California was analyzed for health habits, risk factors, and all-cause mortality (89). The same factors were found to operate also in this population. Frequent consumption of nuts appears protective in that population (1,668 subjects), reducing all-cause mortality in both sexes to 0.6, adjusted for age, smoking, and exercise. In the "old-old" cohort (603 female and male subjects, age ≥ 84 y (90), all-cause mortality was decreased to 0.75 (adjusted multivariate RR 0.82) comparing high to low nut consumption. Mortality from CHD was reduced to 0.55 for high vs. low nut consumption (multivariate adjusted RR 0.61).

A report in 1999 (91) showed that there was a significant protective association between nut consumption and fatal and nonfatal CHD in SDA males and females, RR ~ 0.5 comparing high to low nut consumption, and reduced lifetime risk of CHD by 31% in frequent nut consumers. Results were adjusted for age, sex, smoking, exercise, BMI, hypertension, and consumption of bread, beef, fish, cheese, coffee, legumes, and fruit. Subjects with diabetes were excluded.

The Iowa Women's Health Study (92,93) is a prospective cohort study of postmenopausal females. Prineas reported the

effects of nut consumption on CHD mortality in the 41,837 females enrolled. Predominantly white females, 55–69 y of age, completed a FFQ that asked about the frequency of nut consumption as 1-oz (28 g) portions. Over 5-y, 154 of the 34,484 females free of CHD at baseline died of CHD. Coronary mortality was inversely associated with nut intake, with an adjusted RR of 0.6 for eating nuts 1–3-times a mo, 0.75 for eating nuts once a week, and 0.43 for eating nuts 2–4-times a week, $p = 0.06$.

Kushi et al. (92) reported the results of follow up for 7 y, with 242 females dying of CHD. The primary finding was a strong inverse association of vitamin E consumption from food with the risk of CHD death. Multivariate adjusted RR in the highest quintile, ingesting ≥ 9.64 International Units (IUs) of vitamin E daily, was 0.38. In a subgroup, multivariate analysis of food sources of vitamin E showed the strongest inverse association was with nuts and seeds, exceeding margarine and mayonnaise and creamy salad dressings. RR for the highest quartile of nuts and seeds, $> 4 \times$ /mo, was 0.60, but adjustment for vitamin E reduced this to 0.72, $p = 0.11$. The varieties of nuts consumed were not reported.

Combining the AHS and Iowa Women's Health Study studies (84,85), there seems to be a threshold effect of nut consumption at a frequency of once weekly. An inverse, graded relation was observed between nut consumption and CHD events.

In the NHS (27), of 86,016 female registered nurses (RNs), those consuming at least 5 oz (140 g) of nuts/week had a 35% lowering in nonfatal MI compared with those eating less than 1 oz (28 g) of nuts/mo (rare), RR = 0.65. The RNs ranged in age from 34 to 59 y at baseline. Coronary disease measures included nonfatal MI and fatal CHD. During 14 y of follow-up, 861 cases of MI and 394 deaths occurred. Dietary information was obtained 4 times during the study, and frequency of nut consumption was evaluated for total nuts [later separating peanuts (ground nuts) from tree nuts]. The investigators commented that nut consumption declined over the length of the follow-up period. When peanuts were separated from total nuts, there were few cases of females consuming either peanuts or tree nuts at the highest two quartiles. Multivariate RR for either group was statistically significant (0.79 for other nuts, $p = 0.62$; 0.66 for peanuts, $p = 0.06$). Medical records were reviewed for cases, and the World Health Organization criteria were used for diagnosis.

Hu et al. (94) reported data relating dietary intake of α -linolenic acid and risk of IHD among the females enrolled in the NHS cohort study. A higher intake of α -linolenic acid was associated with an RR of 0.55 in the lowest quintile, p for trend 0.01. The multivariate risk calculation was adjusted for age, standard coronary risk factors (ie, smoking, BMI, hypertension, diabetes, menopausal status, and parental history of premature MI) and dietary intake of α -linoleic acid, alcohol, SF, vitamin C, vitamin E, total energy, and the use of vitamin supplements. The α -linolenic acid intake ranged from 0.71 g/day in the lowest quintile to 1.36 g/day in the highest quintile.

In the Physician's Health Study (95), of 22,000 males followed prospectively for 12 y, as nut consumption increased the risk for cardiac and sudden cardiac deaths decreased significantly. There were 133 sudden cardiac deaths and 449 cardiac deaths. The investigators suggest that after adjustments their data indicate that nut consumption reduces risk of total cardiac deaths and perhaps of sudden cardiac death.

Walnuts and walnut oil

Lavedrine et al. (96) reported a cross-sectional study of a population of farmers in the walnut-producing Grenoble region of France consuming its habitual diet that examined a possible association between walnut consumption (walnut oil and kernels) and blood lipids. In 793 healthy subjects (426 males and 367 females), 18 to 65 y of age (average age ~50 y), the subjects had completed a FFQ covering the previous year. Blood TC, lipoprotein-cholesterol, and apoprotein levels were measured. Increased HDL-C and apo A-1 were associated with walnut consumption, but not TC, LDL-C, or apo B.

The participants attended a health-screening visit with blood sample analysis, filled out a medical questionnaire, and answered the FFQ. The lipid analyses were performed by using standard methods in a laboratory that conformed to national quality assurance plans. The data were analyzed by using multiple linear regression models including possible confounding nutritional and demographic variables. The association was stronger with walnut oil alone than with the oil and kernels. The investigators concluded that there might be a protective effect against CVD of usual walnut and walnut oil consumption by increasing serum levels of HDL-C and apo A-1.

The investigators did not evaluate either fiber or fish consumption or TE intake; however, they did include biomarkers for heart disease risk.

OTHER RELEVANT OBSERVATIONAL STUDIES

Several other observational studies on the epidemiology of CVD may be relevant to the evaluation of the health benefits of walnuts in relation to heart disease, as they addressed the fatty acid components that are found in high concentrations in walnuts.

Leng et al. (97) reported the results of the Edinburgh Artery study that addressed a possible association of plasma triacylglycerol, cholesterol ester, phospholipid, and RBC fatty acid levels with CVD, primarily evaluating peripheral vascular disease. More than 1,100 males and females, 60–80 y of age, were sampled at random. Their health status was assayed by questionnaire and medical records. Diagnosing CVD by using the International Classification of Diseases codes, researchers characterized those subjects having MI (143), stroke (38), or diseases of the lower limb (200). BP in the extremities was measured with a sphygmomanometer and by ultrasonography. Lipids were separated by thin-layer chromatography, and fatty acids were assayed by gas-liquid chromatography. Data were expressed as geometric means. Disease and no-disease categories were compared. Logistic regression was used to develop odds ratios from log-transformation of fatty acid levels.

The results were confusing and difficult to interpret. The triacylglycerol fatty acids showed a significant increase in linoleic acid in patients with MI. RBC phospholipid fatty acids showed that α -linolenic acid was significantly lower in those with stroke and lower-limb disease. The investigators interpreted their data to suggest that α -linolenic acid, but not linoleic acid, was decreased in subjects with stroke, and that 18:3 in the diet might be protective against it. One limitation of this study design is that patients with disease may have modified their diets during the study and that plasma triacylglycerol fatty acids primarily reflect recent dietary intake.

Guallar et al. (26) reported the results of the European Multicenter Case-control Study on Antioxidants, Myocardial Infarction and Breast Cancer (EURAMIC) in eight European countries (Finland, Germany, The Netherlands, Norway, Rus-

sia, Spain, Switzerland, and the United Kingdom) and Israel. The study related adipose tissue n-3 fatty acids with the risk of MI in males. There were 638 cases of first MI recruited within the first week after the event, and 700 matched controls. The α -linolenic acid concentration was significantly lower in the cases than in controls. The adjusted RR of MI for the highest quintile of α -linolenic acid compared to the lowest (0.68) was not statistically significant.

Fatty acids were determined by capillary gas chromatography. The composition should have reflected long-term intake of essential fatty acids, ie, in the previous 2–3 y. However, the investigators did not assess nutrient intake, so that calorie adjustments could not be made. They note the possibility of measurement error in the analysis of components that represent <1% of total fatty acids. They did not measure plasma lipid fatty acids, and referred to other prospective studies [Health Professionals, NHS, and Multiple Risk Factor Intervention Trial (MRFIT) follow-up] (see below), where intake of α -linolenic acid was related inversely to the risk of cardiovascular mortality. The EURAMIC study design did not allow evaluation of sudden cardiac death cases.

Secondary prevention studies of α -linolenic acid

This report of PUFA studies does not include or cite studies with fish or fish oils except in passing and as considered relevant, and focuses only on walnuts, other nuts, or plant sources of PUFA. Simon et al. (98) related the plasma levels of α -linolenic acid to the risk of stroke in a nested case-control study of 96 middle-aged (mean age of 50 y) males in the United States enrolled in the MRFIT study who developed stroke during a 6.9 y follow-up. They were analyzed in relation to 96 matched controls enrolled in MRFIT, a primary prevention trial in males at high risk of developing CHD. The fatty acid composition of cholesterol esters and phospholipids was measured in stored frozen serum samples that were collected at the outset of the study. Data were analyzed by a stepwise conditional logistic regression that controlled for BP and smoking. An increase of one standard deviation (SD) in the serum level of α -linolenic acid in cholesterol esters was related to a 37% reduction of stroke risk. Levels of α -linolenic acid in cholesterol esters were lowered more significantly in stroke case subjects than in controls. The result was not related to differences in BP or blood lipids, and was independent of the risk-enhancing effects of systolic BP and cigarette smoking. The authors commented that sources of α -linolenic acid in the diet are linseed, canola, soy, and walnut oils (intakes not evaluated), and that the protective effect may be related to a reduction in platelet aggregability and blood viscosity.

Another study from the MRFIT (99) found the habitual intake of α -linolenic acid to be 1.688 g (SD 0.736) whereas the habitual intake of linoleic acid was 14.6 g (SD 6.957). The ratio of α -linolenic acid:linoleic acid was associated with a decrease in CVD mortality and all-cause mortality. Dietary intake was analyzed in the usual care (nonintervention) group by 24-h recalls at yearly intervals.

The Lyon Diet Heart Study (28,100) is an intervention trial comparing the “Mediterranean” diet which incorporates α -linolenic acid (219 patients), with a “prudent” diet (192 patients) in secondary prevention of CHD. The diet did not specifically evaluate nuts, but rather added α -linolenic acid in amounts similar to the walnut studies in the form of a canola oil-based margarine and salad dressings. Olive oil was also advised. The reinfarction rate in these MI patients was halved within 27 mo, with similar reductions in cardiac death and nonfatal MI. The investigators also reported an extended

follow-up of 46 mo, with 275 events including evaluation of secondary events and minor events requiring hospitalization. Compared with the control diet, the experimental diet was significantly lower in calories, TF, SFA (8.3% of calories experimental, 11.7% control), linoleic acid (3.6 and 5.3%, respectively), and cholesterol (217 and 318 mg, respectively), and significantly higher in oleic acid and α -linolenic acid (0.81% of calories experimental, 0.27% of calories control).

Adjusted RRs were 0.35 for sudden cardiac death, 0.28 for total primary endpoints, 0.44 for all-cause deaths, 0.33 for total primary and secondary endpoints, and 0.53 for total major and minor endpoints. Results were independent of traditional risk factors, eg, high blood cholesterol, indicating that the experimental diet did not alter the usual relationships between major risk factors and recurrence. The authors suggest a combined approach of a cardioprotective diet with other means (pharmacological) that reduce modifiable risk factors.

The patient's physician prescribed a "prudent" diet. Diet instruction for the experimental group was customized, and the diet was monitored. Plasma fatty acids were analyzed in both groups 2 mo after randomization. Data were analyzed on the intention-to-treat principle. RRs and associations were calculated. Disease outcomes were evaluated in hospitalized patients. Neither the patients nor their physicians were informed that this was a dietary trial. Only plasma α -linolenic acid was associated significantly with an improved prognosis. The profile of plasma fatty acids of the experimental group differed significantly from the control group, with more oleic, less linoleic, more α -linolenic acids and less arachidonic acid, and more EPA. The data for the experimental group derive from 1–4 y follow-up.

MECHANISMS

How the effects of nuts and walnuts reduce cardiovascular risk

This abbreviated discussion of mechanisms is merely illustrative, and is not intended to be comprehensive. Various mechanisms have been postulated in explaining the favorable effect of nuts in reducing CVD risk. Earlier investigations emphasized differences in the fat composition, eg, unsaturation. The results reviewed, however, may go beyond favorably affecting serum lipid and lipoprotein levels and may be manifested rapidly. Changes may occur in lipoprotein composition, independent of concentration. Other mechanisms may involve dietary fiber, NO formation from the increase in arginine in protein, which could influence endothelial function and inhibit platelet aggregation, monocyte adherence, chemotaxis, and vascular smooth muscle cell proliferation. Also, the antioxidant action of vitamin E could reduce LDL oxidation. The content of folic acid could lower the amount of Hcy and perhaps the effect of other phytochemicals such as plant sterols, etc., with possible health benefits that are worth examining.

WALNUTS AS A FOOD

Nuts, including walnuts, are a traditional food in diets from the Mediterranean, South America, and Asia and are ingredients in sauces, stuffing, entrees, snacks, appetizers, and desserts. Walnuts are one of the oldest tree foods known to humans, with historical references dating back to Persia in 7000 BC (101). Ravai (102) has provided data from the Walnut Marketing Board on consumer usage of walnuts in the United States, which include baking (49%), snacks (25%), cooking (11%), salad dressings (11%), and ice cream topping

(4%). Walnuts are stable, especially in the shells. When kept in a cool, dry environment they have a shelf life of 12 mo. Payne (103) used ground walnuts instead of egg protein as whipping agents. Walnuts add and intensify flavor, and lighten foods by fluffing or foaming; they also have rising or puffing properties. Walnuts can be used in making dry cereals, multi-grained breads, cookies, "veggie" burgers, stuffing mixes; walnut paste can be used in salad dressings and can replace croutons in salads.

COMPOSITION OF WALNUTS

Nutrient composition of nuts as a food group

Nuts are very high in fat (73–90% of TE, 48–63 g/100g edible portion) (see Table I). Most nuts are rich in the MUFA, oleic acid, whereas walnuts are high in the PUFAs, linoleic and α -linolenic acids. The dietary fiber content in nuts is high, ranging from 5–9% by weight. Nuts are good sources of arginine-rich protein, potassium, copper, and magnesium. Nuts also are good sources of the antioxidant vitamin E and other compounds with biological activity such as flavonoids, other polyphenols, and sterols.

Walnut composition data

The composition of English walnuts (*Juglans regia* L.) (Table I) is adapted from the database at the U. S. Department of Agriculture, Agricultural Research Service, Nutrient Database for Standard Reference, Release 13, 1999 (104). This high-fat, high-energy food is relatively high in protein, potassium, phosphorus, and folate, with a good content of fiber and vitamin E, and low sugar. Selected values/100 g edible portion include: energy (654 kcal); fat (65.2 g); protein (15.2 g); fiber (6.7 g); phosphorus (346 mg); potassium (441 mg); folate (98 μ g); and vitamin E (2.9 mg). The lipid content consists of SFAs (6.1 g), predominantly palmitic (4.4 g) and stearic (1.7 g) acids; MUFAs (8.9 g) almost entirely oleate; and PUFAs (47.2 g), predominantly linoleic (38.1 g) and α -linolenic (9.1 g) acids. There are 72 mg of phytosterols. The amino acid composition is highest in glutamic acid (2.8 g), arginine (2.3 g), aspartic acid (1.8 g), leucine (1.2 g), serine (0.93 g), glycine (0.82 g), valine (0.75 g), phenylalanine (0.71 g), alanine (0.7 g), proline (0.7 g), isoleucine (0.62 g), and threonine (0.6 g). The concentrations of methionine and cystine are both low. A study by Pennington (96) contains comprehensive data on the nutrient content of individual nuts including English and black walnuts (*J. nigra* L.).

Lipids

Zwarts et al. (105) provided data on the fatty acid composition of walnuts (oil extracted from walnut kernels of *J. regia* L.) from ten different New Zealand, European, and United States commercial cultivars collected over two growing seasons and found a distinctive fatty acid profile. The total oil content ranged from 62.4 to 68.7%. Oleic acid (18:1 n-9) content ranged from 14.3 to 26.1% of the total fatty acids, whereas the linoleic acid (18:2 n-6) content ranged from 49.3 to 62.3% and the linolenic acid (18:3 n-3) from 8.0 to 13.8%. Flavor stability was affected by shelf life. The PUFA content of walnut oil as a percent of total fatty acids also can vary from 47% in France to 81% in the Ashley cultivar from California (106). Some walnut oils have been reported to contain up to 90% PUFA. Glycolipids have been examined in walnuts (107). The sugar was exclusively galactose. The lipid distribution was similar to that in rice bran oil and in soybeans. The

sterol was predominantly β -sitosterol. The essential volatile oils in walnuts that are rich in aliphatic hydrocarbons have been quantified (108).

The tocopherols in walnuts, in contrast to other nuts, are uniquely enriched in γ -tocopherol (109). The authors analyzed the tocopherols in French and United States walnuts and found that α -tocopherol ranged from 1.08 to 4.05, γ -tocopherol from 21.8 to 26.5, and δ -tocopherol from 2.51 to 4.53 mg/100 g. Levels declined about 30% after 3 mo of refrigeration.

Fiber

Cardozo and Li (110) analyzed eight varieties of nuts for total dietary fiber content. Macadamia nuts, with 14.87 g/100 g were highest in fiber followed by walnuts (9.79 g/100 g) that, in turn, exceeded almonds (9.11 g/100 g) and pecans (9.00 g/100 g). Cashews, peanuts, and pistachios were lower in fiber (3.91, 6.23, and 6.98 g/100 g, respectively). Nuts, including walnuts, are high in phytate (111). Walnuts contain 982 mg/100 g, a value similar to almonds (1,138 mg) and roasted peanuts (952 mg), but higher than hazelnuts (648 mg) and lower than Brazil nuts (1,719 mg).

Minor components

Other phytochemicals that may be biologically active in walnuts that have been quantitated in small amounts and studied include n-alkanes (which are low in walnut oil) (112), tannins (111,113), and juglone, a naphthoquinone with antimicrobial activity (114). Macfarlane's analysis of walnuts showed them to contain >1,500 mg polyphenols/100 g, a value more than double that of the other nuts tested.

Possible adverse effects of walnut components

Lipid peroxidation. Intake of MUFA relative to diets rich in PUFA may increase the resistance of LDL to in vitro peroxidation (74), although data on the effect of PUFA on LDL oxidation are conflicting. Relevant studies include the Barcelona Walnut trial (81,115), which showed no increase in oxidative stress with walnuts as a source of increased PUFA, and Berry's Jerusalem Nutrition Study (83), which included walnuts in the high-PUFA diet and showed an increase in oxidative stress. Reaven et al. (116) reported at the same time that liquid formula diets enriched in MUFA (85% of fatty acids as oleate) had decreased rate of formation of CDs as compared to diets enriched with PUFA (60% as linoleate), with no difference in TBARS, and an increase in macrophage degradation. This 39% fat diet was fed to five (MUFA) and four (PUFA) subjects. The liquid formulas were equal in vitamin E concentration (which might have a protective effect against oxidative stress if increased in relation to PUFA, as in walnuts). This study appeared to provide about 2.5-times the amount of linoleate as the Walnut clinical intervention trials.

Binding of iron. Macfarlane et al. (111) noted that 50 g nut meal paste in a sandwich markedly reduced iron absorption; this was reversible by 50 mg ascorbic acid. This reduction was similar to effects of soy protein and wheat bran, whereas ascorbic acid had only minor effects to improve iron absorption with these foods. Effects were ascribed primarily to the content of phytate and polyphenols. Nuts included walnuts, almonds, peanuts, hazelnuts, and Brazil nuts, all of which inhibit iron absorption, and coconuts, which had no effect.

CONTROLLED INTERVENTION STUDIES WITH NUTS OTHER THAN WALNUTS

Almonds

Spiller et al. (117) reported the effects of ingesting 100 g almonds (~600 kcal) on serum lipids in hypercholesterolemic subjects. Groups of 15 subjects each, mostly females, were randomized to either the almond-based diet, an olive oil-based diet, or, as a control, a dairy-based primary source of fat. The study lasted 4 weeks, with a 1-week run-in period; there was no cross over. Significant decreases in TC, LDL-C, and the LDL:HDL ratio were observed on the almond-based diet with no change in HDL-C. No changes were observed with the olive oil diet, whereas TC and HDL-C increased significantly with the cheese and butter diet. All subjects were prescribed a similar background diet (BD) and provided many foods. The almond diet was lower in cholesterol than the other diets. BW did not change. Duplicate samples for lipid analyses were obtained along with 3-day food records. Dietary compliance was monitored with random 24-h telephone recalls. Lipid analytic procedures were standardized with quality controls. Data analyses were by ANOVA.

Subjects ingesting the almond diet consumed more energy as fat (39 vs. 35%), while the olive oil group ingested the most calories. The almond diet was high in MUFA, lowest in SFA, and highest in PUFA. The authors suggested that further studies on nut fibers be carried out.

Sabaté et al. (118) reported a metabolic feeding study in 25 normocholesterolemic males and females with an almond diet replacing 20% of TE in a Step 1 diet, each fed for 4 weeks in a crossover design. Both diets lowered TC and LDL-C in comparison to baseline, with the almond diet further decreasing these parameters and triacylglycerol 4–6% in comparison with the Step 1 diet. The authors did not indicate if these results were statistically significant and did not provide the data for these measurements. HDL-C was unchanged and the LDL:HDL ratio decreased on the almond diet. Fasting glucose significantly decreased on the almond diet.

Macadamia nuts

The study by Curb et al. (119) was a well-controlled, randomized, crossover trial carried out in healthy, young-to-middle-aged males and females with normal lipids or with hypercholesterolemia. The investigators compared a "typical American" diet with 37% of energy from fat (16% SFA, 14% MUFA, and 7% PUFA) with an American Heart Association Step 1 diet (30% fat, 9% SFA, 14% MUFA, and 7% PUFA), and a macadamia nut, MUFA-enriched diet (37% fat, 9% SFA, 21% MUFA, and 7% PUFA). Both modified diets improved the lipid profile significantly compared with the "typical American" diet.

Finely ground macadamia nuts were used as ingredients in other foods. Although both modified diets had similar lowering effects on TC and LDL-C, the macadamia diet lowered triacylglycerol, whereas triacylglycerol increased with the Step 1 diet. Both diets lowered HDL-C by about 4%. The results suggest the lipid-lowering benefits of an alternative to the "step 1 diet" reduction in TF and SF. Instead, the investigators suggested substituting sources of MUFA, such as nuts, for some SFA.

Macadamia nuts, like other nuts, are high in fat (75% by weight) with oleic acid predominating, which is similar to the contents in almonds and peanuts. Macadamias, however, have a large amount of a less common MUFA, palmitoleic acid (16:1). This fatty acid has not been compared with the more

common long-chain fatty acids, so that possible benefits (or adverse effects) of enrichment with this fatty acid are unknown. This study did not include measurements of other lipid and lipoprotein risk factors such as the apoproteins, or the subclasses of HDL or LDL, or Lp (a) that are emerging as important predictors of CHD. The authors did not provide information about the exact amount of nuts eaten daily, nor the foods in which they were incorporated, nor meal plans, menus, or recipes. In a pilot study, investigators reported that subjects ingested 45-90 g of nuts daily. The subjects probably consumed from 45-50 g of nuts as whole nuts, nut meal, and other products, which was about one-third of their daily fat intake.

A related study from Australia (120) reported a crossover, randomized trial in males ($n = 7$) and females ($n = 7$) comparing a high-MUFA (42% fat) diet incorporating macadamia nuts with a 20% LF diet high in complex carbohydrates. The data showed significant lowering of TC and LDL-C with both diets. Triacylglycerol decreased and there was no change in HDL-C with the macadamia diet in contrast to lowering of HDL-C with the LF diet. In this study, 25-50 nuts were consumed daily, added to salads and desserts, or as snacks. The investigators analyzed the fatty acid composition of the nuts: 0.8% myristic (14:0); 8% palmitic (16:0); 19% palmitoleic (16:1); 4% stearic (18:0); 56% oleic (18:1); 2% linoleic (18:2); 3% arachidic (20:0); and 3% gadoleic (20:1). Some studies with palmitoleic acid from macadamia oil suggested favorable properties beyond those of oleic acid in preventing CVD. They suggest incorporating macadamia nuts in cereal mixes, spreads, flavorings, and ice cream.

Tung et al. (121) reported the results of a randomized, crossover study of a macadamia nut-based diet and a "typical American" diet. Both diets contained 38% fat, with the macadamia diet lower in SFA (9%) and higher in MUFA (22%); the control diet had 20% SFA and 11% MUFA. Twenty-two healthy male and female volunteers, 20-52 y of age, were fed each diet for 8 weeks, with a 2-mo washout period. TC and LDL-C were significantly lower with the macadamia diet, and large particle LDL₃ (a fraction of LDL-C) also decreased along with mean LDL particle concentration and particle size. The latter changes would adversely affect the risk of CVD.

Pistachio nuts

Edwards et al. (122) used a random, crossover design and reported the effects in 10 healthy, moderately hypercholesterolemic males and females of adding pistachio nuts as snacks, 100 g providing 20% of calories, during a 3-week intervention. Comparing the pistachio diet with the regular diet, researchers found that TC, TC:HDL-C, and LDL-C:HDL-C decreased significantly, with no significant changes in LDL-C, HDL-C, or triacylglycerol. BW and BP were unchanged. With the pistachio diet, SFA decreased and MUFA, PUFA, and fiber increased significantly.

Pecans

Rajaram et al. (123) reported the effects of a pecan-rich diet on serum lipids and lipoproteins in 23 normocholesterolemic males and females. After a run-in period of 2 weeks on a "typical American" diet, subjects were fed for 4 weeks either the pecan diet or a Step 1 diet in a randomized, controlled, crossover design. The pecan diet was higher in TF (42 vs. 30%); both diets had 8% SFA whereas the pecan diet was higher in MUFA (24 vs. 15%) and PUFA (10 vs. 7%). The pecan diet significantly decreased serum cholesterol, LDL-C,

triacylglycerol, apo B, and Lp (a) levels and increased HDL-C and apo A-1 levels compared with the Step 1 diet.

Other data from this study, reported by Haddad et al. (124), showed that the pecan diet increased stool fat excretion. Stools were collected for 48 h in six subjects during each of the diet periods. Stool fat (25 g pecan, 6 g Step 1) and the percentage of fat in the stool (8% pecan, 3% Step 1) increased significantly with the pecan diet. The authors suggest that these changes may explain, in part, why the added fat or calories in a nut-based diet do not lead to weight gain. The pecan fat may not have been as well-absorbed because of the structure of lipid-storing granules in nuts or the fiber components of the nuts.

Peanuts

Peanuts are ground nuts or legumes, rather than tree nuts, which is the category that includes the varieties of nuts already described. Nonetheless, consumers perceive them as nuts and eat them as snacks and use them as condiments. Their consumption in the United States exceeds that of tree nuts. The fatty acid composition of peanuts resembles most tree nuts, ie, they contain primarily MUFAs. Peanuts, however, are uniquely enriched in very long-chain SFAs and contain plant proteins rich in arginine and phytochemicals.

Peanut oil has been studied extensively in relation to experimental atherosclerosis. Results are controversial in terms of the atherogenicity of peanut oil, in part depending on the species tested.

Since peanuts are widely consumed, and peanut oil is a major fat source in many populations, this report summarizes the data from recent controlled studies relating the consumption of peanuts to the risk of heart disease.

Kris-Etherton and collaborators (125) compared the lipid profile of a high-MUFA diet of peanuts and peanut butter providing one-half the fat, with two other high-MUFA diets—olive oil and peanut oil—a Step 2 American Heart Association diet, and an "average American" diet (AAD) with 34% TF. The study was randomized, double-blind, and had five crossover periods. Twenty-two healthy, normocholesterolemic males and females were fed each diet for 24 day, with 4-to-11-day breaks between periods. All meals were provided. The three high-MUFA diets, equal in TF to the AAD (34%), were lower in SFA (7 vs. 16%) and cholesterol (200 vs. 400 mg); the Step 2 diet was 25% fat. The MUFA and Step 2 diets were similar in their lowering of TC and LDL-C compared with the AAD. Triacylglycerol concentrations were lower with the MUFA diets and increased with the Step 2 diet; HDL-C was unchanged with the MUFA diet and lower with the Step 2 diet. Apo A-1 and apo B and the ratios of TC:HDL-C and LDL-C:HDL-C were lower with the MUFA diets. Standard lipid methods were used to assay plasma lipids and lipoproteins and diet fatty acid composition, with quality control. Data, which were analyzed by ANOVA, showed no appreciable differences among the MUFA diets. The authors concluded that a high-MUFA, cholesterol-lowering diet that includes peanuts and nuts is an acceptable dietary approach toward favorably affecting CVD risk status.

O'Byrne et al. (126,127) described studies with high-oleic peanuts fed for 6 mo that evaluated effects on serum lipids, lipoproteins, apoproteins, and lipoprotein oxidation. In one study, subjects were older, healthy hypercholesterolemic females. The high-oleic peanut cultivar contains 76-80% of fat as MUFA. Prepackaged daily rations of dry roasted peanuts were provided, and subjects consumed 35-68 g daily. LDL fatty acid composition and oxidation were measured in five

subjects eating the peanut diet (26% fat, 14% MUFA) and in six subjects following a LF diet. The LDL oxidation parameters (viz., CD and lipid peroxidation) were improved with the peanut diet.

In groups of 12 and 13 females, in which each ate the peanut and LF diets, TC and LDL-C decreased and the proportion of larger, lipid-rich, less atherogenic LDL increased with the peanuts. Serum triacylglycerol, HDL-C, and apo A-1 levels were unchanged.

Combinations of nuts

Jenkins et al. (128) reported effects on serum lipids of a diet high in nuts (60–100 g almonds, cashews, and peanuts) in a 2-week random, crossover-designed study of 10 healthy, young, normolipidemic Canadian males and females. The nuts were included in a plant-based diet high in green, leafy vegetables and fruits and low in fat (25% of TE). The control diet was their HD (fat intake 29% of TE). Significant differences observed with the plant-based diet were decreases in LDL-C and the ratios of TC:HDL-C, apo B:A-1, and Lp (a).

When the control period preceded the plant-based diet, there was no washout. When the vegetable diet was given first, the investigators waited 5 mo to study the HD in order to avoid carry-over habits from that period. Nuts were provided to the subjects. Samples for lipid analysis were drawn once per treatment period and analyzed by validated methods. Data of percent change in variables were analyzed by *t* test, multiple regressions, and correlation coefficients. The vegetable diet was significantly lower in SFA and cholesterol and higher in fiber compared to the control diet.

The study was limited by small sample size and short duration. The diet not only was enriched in nuts, but eliminated animal products, and so may not easily be extrapolated to less extreme dietary modifications. The investigators noted, however, that the observed changes in LDL-C were double that predicted from the change in fatty acid content.

A related study from India reported effects of a vegetarian diet on the outcome of acute MI during a 6-week follow-up (129). Immediately after a MI, patients were randomized to a vegetarian diet which included fruits, vegetables, almonds, and walnuts (204 patients) or their physician's prescribed "prudent" diet (202 patients). The lipid profile improved on the vegetarian diet with decreases in TC, LDL-C, and triacylglycerol, and there was a 34% decrease in total cardiac endpoints. The diet was significantly lower than the diet prescription in energy, SFA, cholesterol, caffeine, and salt, and increased in fiber, vegetable protein, vitamins C and E, magnesium, potassium, copper, selenium, and chromium.

This study might indicate the value of a plant-based diet that includes walnuts in the early management of acute MI and could be compared with studies reviewed on nutritional factors in relation to cardiac arrhythmias or thrombosis.

These clinical human dietary intervention studies are summarized in Table 2.

PROTEINS IN NUTS AND AS A FACTOR IN HEART DISEASE RISK

At present, conclusive evidence of a consistent effect of protein on the risk of heart disease appears limited to soy protein. The studies showing the hypocholesterolemic effects of soy proteins in man and animals are not reviewed. A health benefit claim for soy protein and lipid-lowering to reduce heart disease was authorized by the FDA (78).

Plant proteins differ in their amino acid composition from animal proteins; they are enriched in arginine and low in lysine. A diet rich in plant proteins (eg, plant-based, vegetarian), in contrast to animal proteins (eg, casein, beef), reduces the risk of atherosclerosis, in part by favorably influencing the lipid profile. The ratio of lysine:arginine was implicated in hypercholesterolemia and atherosclerosis in earlier animal studies (130,131). More recently, with the evolution of our understanding of the significance of NO in vascular biology, arginine emerges with new importance as the precursor of NO.

Many studies support the hypothesis that plant proteins, and specifically arginine or the low lysine:arginine ratio, reduce experimental atherosclerosis. The investigators implicated mechanisms related to lipids, lipoproteins, lipid oxidation, stimulation of the vascular endothelium, and the effects on coagulation (platelet aggregation) mediated by prostaglandins and cytokines, as well as on arrhythmias. Studies with arginine investigating reduction of CVD mortality and morbidity report beneficial effects on heart failure, BP, and stroke. Studies have administered L-arginine, arginine-enriched proteins, nut-enriched diets and, specifically, walnut-enriched diets. The lysine:arginine ratio of walnuts is low (0.16). Soy protein, for example, has a ratio of 0.58–1, with animal proteins like casein or whole milk having ratios of 1.89 and 2.44, respectively (131).

Animal studies

Kritchevsky (131) observed that increasing arginine in the diet of hyperlipidemic rabbits or, more importantly, the low lysine:arginine ratio, had an antiatherogenic effect. Although the source of increased lysine:arginine ratio was fish protein (ratio of 1.44) and the investigators were unable to provide a mechanism for their results, the antiatherogenic effect has been extrapolated to later studies that used diets enriched in plant proteins with low ratios of lysine:arginine, or a plant source of arginine.

Ravel et al. (132) studied the effect of walnut meal compared to soybean meal on hypercholesterolemia and growth rate in female rabbits. The hypercholesterolemia, which is correlated with hypertriglyceridemia, was reduced after 6 weeks on the walnut diet but the mechanism was not elucidated. The surviving animals had a net gain in BW of 10%.

Ravel et al. (133) studied walnut meal as a source of arginine in feeding rabbits a semisynthetic diet compared with casein or soy protein diets. The walnut diet showed the greatest decrease in platelet aggregation and a decrease in TC. Platelet aggregability correlated best with the lysine:arginine (0.16 walnuts, 0.70 soybeans, and 1.54 casein) and the HDL:LDL ratios. The investigators proposed that an increase in the ratio 20:5 n-3:20:4 n-6 in platelets may have a favorable influence on their aggregation. This laboratory had published data that showed the hypocholesterolemic effect of vegetable proteins with significant lowering of LDL-C and the beneficial effect on platelet aggregability (134). LDL-C has been shown to increase platelet aggregation and HDL-C to lower platelet reactivity.

Confirming the antiatherogenic effect in rabbits, Aji et al. (135) reported that arginine prevents xanthomas and inhibits atherosclerosis in an LDL-receptor knockout mouse model of familial hypercholesterolemia. Mice were fed a high-cholesterol diet with or without arginine or with arginine and an inhibitor of nitric oxide synthase (NOS). A control group was fed a standard mouse laboratory stock diet. Arginine (2.25%)

was added to the drinking water for 6 mo. All mice fed the high-cholesterol diet developed xanthomas, but none of the arginine-supplemented mice did. The size of aortic atherosclerotic lesions was reduced significantly in the arginine group compared with the mice fed the unsupplemented, high-cholesterol diet. The investigators concluded that the antiatherogenic effect of arginine was mediated by stimulating NOS and suggested that L-arginine might be helpful in treating familial hypercholesterolemia. In all groups fed the high-cholesterol diet, ie, with or without arginine or arginine and the NOS inhibitor, there were no differences in plasma cholesterol levels or in the distribution of cholesterol among atherogenic lipoprotein.

Human studies

Adams et al. (136) showed that increasing the intake of L-arginine improved platelet function markedly, but did not affect vascular reactivity. They administered 7 g arginine powder taken with meals 3 times/day for 3 day in a randomized, placebo-controlled, crossover study, with a washout period of 7–14 day. In the study subjects (12 healthy, young males), plasma arginine levels were about 2.5 times higher and platelet arginine increased seven-fold. The arginine intake was estimated to be about 4 times greater than the normal arginine daily intake that had been estimated in Australia at about 4 g per day in 1984. The inhibition of platelet aggregation correlated significantly with the plasma level of arginine. They interpreted their experimental data as showing a relatively platelet-specific effect of arginine in this brief period of oral administration to increase NO production locally but not systemically, and with no changes in vascular hemodynamics or plasma lipids. Although the authors indicated that they calculated LDL-C by using the Friedewald equation, they present numbers that indicate that they simply subtracted HDL-C from TC, even though they had data of triacylglycerol measurements to use in the calculation.

A randomized, double-blind, placebo-controlled study by Wolf et al. (137) reported effects of feeding 8.6 g L-arginine for 2 weeks to 23 hypercholesterolemic subjects (17 males and 6 females). The study used a parallel design. These subjects were also compared with 14 normocholesterolemic control subjects (13 males and 1 female). Arginine was administered as capsules containing 700 mg; three capsules were taken 4 times daily. This regimen increased arginine intake about 50% compared to the 5.6 g reported as the usual daily intake in the United States in 1986. Plasma arginine increased 55%. Platelet aggregability increased moderately in hypercholesterolemic subjects compared with the normal control and improved modestly with arginine. The authors noted that endothelial vasodilator function is depressed in hypercholesterolemia due to increased degradation and/or reduced synthesis of NO. The abnormality can be corrected by administering L-arginine, which is then converted to NO. Future studies should address whether L-arginine, via its antiplatelet effects, will reduce vascular thrombosis and the progression of CVD.

Rector et al. (138) reported a trial of arginine supplementation in heart failure. They compared the effects of arginine, 5.6–12.6 g daily for 6 weeks with a placebo (double-blind). The subjects were heart-failure patients (14 males and 1 female, mean age 56 y). With arginine, blood flow in the forearm and distance walked increased, an adverse functional score declined, arterial compliance increased, and circulating endothelin decreased. Plasma arginine increased about 15%.

These studies of arginine feeding in human subjects reported neither the diet of their subjects nor its arginine con-

tent. It would be of interest to know the current intake of arginine in the United States in order to optimize arginine intake. It remains to be elucidated whether arginine supplementation is a benefit if it is added to a vegetarian diet and to determine if there is a way to enrich the arginine content in the walnut crop.

Arginine studies are exciting in terms of possible effects on clinical manifestations of heart disease and mortality. Note that walnuts are especially rich in arginine (Table I).

Nitric oxide and vascular function and disease

This topic was reviewed in an editorial in the *New England Journal of Medicine* (139). L-arginine is converted to L-citrulline and NO in a reaction catalyzed by NOS. Several isoforms of the enzyme are located in endothelium, macrophages, and neurons that respond variably to agonists and calcium induction. Exogenous arginine enhances NO synthesis. NO determines resting vascular tone and stimulates thrombin, adenosine diphosphate, and bradykinin; it is involved in shear stress and cyclic strain. NO induces smooth muscle cell relaxation by activating guanylate cyclase to produce cyclic guanosine monophosphate, the putative second messenger of NO. Endogenous NO determines the pressor response to sodium. NO also inhibits platelet adhesion, activation, and aggregation, and is antithrombotic. Other actions of NO include inhibition of growth of vascular SMCs. L-arginine administration in experimental animals may inhibit neointimal vascular proliferation of the aorta after balloon injury.

Other discussions on NO (135,137) and an editorial by de Lorgeril (140) consider the effects of NO on BP. Additional actions of NO include the inhibition of monocyte adhesion to endothelial cells and expression of adhesion molecules, decreased expression of monocyte chemotactic proteins, and scavenging by superoxide radicals. These may reduce LDL oxidation and expression of redox-sensitive adhesion molecule genes. In hypercholesterolemic animals and humans, endothelial vasodilatation is depressed, either from increased degradation and/or reduced synthesis of NO. This abnormality can be prevented by administering L-arginine. Generating NO in vivo from blood vessels has been demonstrated. The relation of NO and arginine to eicosanoid production remains to be discovered.

De Lorgeril (140) also discussed some paradoxes. Beef, pork, and lamb are high sources of arginine, albeit also high in SFAs, as are chicken and fish, foods that are recommended in diets to prevent heart disease. The n-3 fatty acids in fish have been related to increases in NO excretion, presumably by stimulating NO synthesis. This implies that the combination of n-3 fatty acids and arginine, with low SFAs and zero cholesterol, as in nuts, should be effective in cardioprevention diets. Emphasizing nut intake also may favorably affect Hcy levels that are lowered by folate (folate content is high in walnuts) and increased by methionine, which is low in nuts and high in meats. Since the vascular toxicity of Hcy may be related in part to inhibition of NO, increasing arginine consumption (by eating nuts) also improves the folic acid:methionine ratio and may have interrelated effects in preventing CVD independent of effects on lipids. Manipulation of the diet with foods in order to lower the risk for heart disease may be safer, less costly, and more acceptable to those at risk, perhaps delaying pharmacologic interventions, or at least decreasing the doses of drugs.

OVERVIEW AND SUMMARY OF CLINICAL TRIALS

The Walnut trials have, in aggregate, involved ~200 healthy, young- to -middle-aged subjects, mostly Caucasian, about two-thirds of whom were males, tested usually for 4 to 6 weeks. TC and LDL-C have ranged from normal to moderately hypercholesterolemic. Some subjects have been overweight. The subjects are representative of the 51% of the adult population in the United States who are at higher risk of CHD. Daily intake of walnuts in these trials ranged from 48 to 84 g. Outcome variables have included serum plasma lipids, lipoproteins, lipid and lipoprotein classes and subclasses, apoproteins, and oxidative stress. The diet has been monitored variously by recall, food records, FFQ, or all foods were provided. Subjects have been studied under metabolic ward conditions or free-living. Analyses have been made of the fatty acid composition of the diet, plasma, lipid classes, or adipose tissue. Data analyses have used *t* tests, ANOVA measurements, linear modeling, and stepwise conditional logistic regression methods. The number of subjects enrolled in these trials has ranged from 16 to 49.

Data have uniformly shown significant lowering of TC, LDL-C, and the ratio LDL-C:HDL-C. Apoprotein response has been variable. Changes in lipid particle size were assayed. Cholesterol was decreased significantly in IDL and redistributed from the more atherogenic small, dense LDL to larger LDL particles. Adding walnuts to diet was associated with significant shifts in cholesterol that redistributed it from larger into smaller HDL particles. Lipid particle changes occurred in the absence of lipid lowering, suggesting a favorable anti-atherogenic effect of walnuts that is independent of any changes in circulating lipid levels. The fatty acid composition of plasma or RBC membranes, with increased PUFA, indicates that the subjects complied with the various diets.

Studies have rarely included patients with CHD. Evaluating cardiovascular outcomes would require more time and involve more subjects of both sexes. Some investigators had difficulty controlling fat and energy intake with the walnut diet. They observed that net BW did not increase; however this finding requires explanation. Data concerning oxidative stress were inconclusive, as one study showed an adverse effect and another reported no change.

WEIGHT OF EVIDENCE

In evaluating the scientific evidence to support a health benefit claim for walnuts and CHD, the author of this LSRO report and the Expert Panel of reviewers followed the approach in the FDA "Guide for Review of Health Claims" regarding the significant scientific agreement standard about the relationship between a food substance and a disease or health-related condition (141).

Specifically, the FDA document notes that: "... the assessment of significant scientific agreement then derives from the conclusion that there is a sufficient body of sound, relevant scientific evidence that shows consistency across different studies and among different researchers and permits the key determination of whether a change in the dietary intake of the substance will result in a change in a disease endpoint. ..."

Further, "... significant scientific agreement does not require a consensus or agreement based on unanimous and incontrovertible scientific opinion. However, on the continuum of scientific discovery that extends from emerging evi-

dence to consensus, it represents an area on the continuum that lies closer to the latter than to the former."

In the context of the foregoing, interventional studies on human subjects were accorded the greatest scientific weight in reaching the conclusions presented here. The studies were well designed, executed, and controlled. The human subjects were representative of the 51% of the adult population of the United States at higher risk of CHD. The observational studies were supportive because walnuts were not always identified as an independent variable, and the data collection was semi-quantitative.

Studies on tree and ground nuts other than walnuts were included in the scientific evaluation for completeness and to complement the findings reported in the observational studies. Similarly, abstracts were included to round out the literature, with the caveat that the evidence presented had not been peer-reviewed and could not be weighed. Moreover, none of the conclusions in this report is based on the abstracts cited.

CONCLUSIONS

1. Walnuts, as part of a heart-healthy diet, lower blood cholesterol concentrations in humans and animals.
2. Walnuts are unique compared to other nuts because they are predominant in n-6 (linoleate) and n-3 (linolenate) polyunsaturated fatty acids rather than the monounsaturated fatty acids that are present in most other nuts. Walnuts, as do other nuts, have a high fat content, but are low in saturated fatty acids. These fats are similar to liquid vegetable oils and margarines made from liquid vegetable oils.
3. Walnuts as a food contain multiple health-beneficial components, viz., having a low lysine:arginine ratio and high levels of vitamin E, arginine, folate, fiber, tannins, and polyphenols.
4. Walnuts have a long dietary history and continue to be readily acceptable as part of the daily diet. The clinical dietary intervention studies show that consuming walnuts does not cause a net gain in body weight when they are eaten as a replacement food.
5. The supporting human clinical walnut intervention studies suggest reduced relative risk of coronary heart disease, yet they are inconclusive because there have been only five controlled, peer-reviewed, published trials with few subjects. There are few trials of extended duration essential for critical evaluation of the sustainability of the health-beneficial outcomes and evidence of adverse effects (eg, body weight gain and gastrointestinal intolerance). The subjects, though, were representative of the 51% of the adult population in the United States at higher risk of coronary heart disease. The existing studies, considered in their totality, suggest that walnuts, as part of a heart-healthy diet, lower blood cholesterol concentrations. This strong trend needs to be substantiated.
6. The several large human prospective observational studies, along with their respective subpopulation cohorts, all demonstrated a dose response-related inverse association of the relative risk of coronary heart disease and coronary vascular disease with the frequent daily consumption of small amounts of nuts, including walnuts. This same response outcome is upheld in different population groups (males, females, African-American, the oldest-old and patients with coronary heart disease) for all-cause mortality, with a 30–50% decreased relative risk of coronary heart disease reported.

TABLE 1

Composition of nuts (per 100 g edible portion)

	Protein	Total lipid	Carbohydrate (by difference)	Fiber (total dietary)	Minerals					Vitamins	Amino acids				
					Iron	Magnesium	Zinc	Copper	Selenium	Vitamin E	Lysine	Arginine			
					<i>g</i>				<i>mg</i>			<i>µg</i>	<i>mg</i>	<i>g</i>	
Walnuts	15.23	65.21	13.71	6.7	2.91	158	3.09	1.586	4.6	2.942	0.424	2.278			
Almonds	21.26	50.64	19.74	11.8	4.29	275	3.36	1.110	7.9	26.179	0.601	2.466			
Brazil Nuts	14.34	66.22	12.80	5.4	3.40	225	4.59	1.770	2960	7.600	0.541	2.39			
Coconut meat	3.33	33.49	15.23	9	2.43	32	1.10	0.435	10.1	0.730	0.147	0.546			
Hazelnuts	14.95	60.75	16.70	9.7	4.70	163	2.45	1.725	4.0	15.188	0.42	2.211			
Macadamia Nuts	7.91	75.77	13.82	8.6	3.69	130	1.30	0.756	3.6	0.537	0.018	1.402			
Peanuts	25.8	49.24	16.14	8.5	4.58	168	3.27	1.144	7.2	9.130	0.926	3.085			
Pistachios	20.48	43.19	29.19	10	4.27	121	2.20	1.300	7.0	4.600	1.162	2.049			
Fatty acids, saturated															
	Total	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	20:0	22:0	17:0	24:0		
	<i>g</i>														
Walnuts	6.126	0	0	0	0	0	0	4.404	1.659	0.063					
Almonds	3.881	0	0	0	0	0	0	3.198	0.683						
Brazil Nuts	16.154	0	0	0	0	0	0.620	9.519	5.679						
Coconut meat	29.698	0	0.191	2.346	1.864	14.858	5.866	2.839	1.734						
Hazelnuts	4.464	0	0	0	0	0	0	3.097	1.265	0.102					
Macadamia Nuts	12.061	0	0	0	0	0.076	0.659	6.036	2.329	1.940	0.616	0.124	0.281		
Peanuts	6.834	0	0	0	0	0	0.025	5.154	1.1						
Pistachios	5.286	0	0	0	0	0	0	4.751	0.453	0.041	0.041				
Fatty acids, monounsaturated															
	Total	16:1	18:1	20:1	22:1	Fatty acids, polyunsaturated							Cholesterol	Phytosterols	
	<i>g</i>														
Walnuts	8.933	0	8.799	0.134	0	47.176	38.095	9.081	0	0	0	0	0	0	72
Almonds	32.156	0.234	31.923	0	0	12.214	12.214	0	0	0	0	0	0	0	120
Brazil Nuts	23.016	0.324	22.382	0.062	0	24.129	23.807	0.062	0	0	0	0	0	0	*
Coconut meat	1.425	0	1.425	0	0	0.366	0.366	0	0	0	0	0	0	0	47
Hazelnuts	45.674	0.137	45.406	0.131	0	7.92	7.833	0.087	0	0	0	0	0	0	96
Macadamia Nuts	58.878	12.981	43.756	1.890	0.233	1.502	1.296	0.206	0	0	0	0	0	0	116
Peanuts	24.429	0.009	23.756	0.661	0	15.559	15.555	0.003	0	0	0	0	0	0	220
Pistachios	22.665	0.450	22.049	0.165	0	13.077	12.831	0.247	0	0	0	0	0	0	214

* No data.

TABLE 2
Human clinical and animal intervention studies

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Almario et al. (82)	13 postmenopausal F and 5 M (60 ± 8 y); fasting triacylglycerol >2.26 mmol/L (200 mg/dL); TC >5.17 mmol/L (200 mg/dL); and LDL >3.36 mmol/L (130 mg/dL). Effects of walnut intake on plasma fatty acids, LPs, and lipoprotein-subclasses in patient with combined hyperlipidemia on background of both HD and restricted dietary fat intakes. Sequential, crossover study.	Subjects sequentially followed: I. HD (4 wk), II. HD + walnuts (48 g walnuts + 8,460 kJ energy intake/d) (6 wk), III. LF diet (20% fat) (6 wk), IV. LF diet + walnuts (6 wk). Daily walnut supplementation was given in pre-weighed packages. During each intervention period, 7-d food records were obtained. In middle and at end of each dietary period, subjects were seen in the morning and after an overnight fast. They were weighed and had blood samples drawn. Samples collected at end of each period were used for statistical analysis.	Diets II and IV did not cause weight \uparrow despite \uparrow intakes of energy and fat. Triacylglycerol did not change. TC, LDL, and apo B did not \uparrow . Lipid profile may not have changed because patient had combined hyperlipidemia and their HD had less TF and SFA, and more MUFA, as compared with general population. Placed next on diet IV, TC, and LDL \downarrow significantly. Addition of PUFA in diet IV may have specific actions on LP metabolism. In support of this idea, even when plasma lipid concentrations did not change, particle size and composition of lipoprotein did. The most atherogenic lipoprotein in the plasma are IDL and small, dense LDL. On diet I, 13% of LDL was in IDL-size particles. Patients with combined hyperlipidemia had detectable IDL in fasting plasma. On diet II, plasma IDL \downarrow by 31%. On diet III, IDL \uparrow by 19%. Walnut intake also exerted favorable effects on LDL. Diet II significantly \uparrow cholesterol in the large LDL and \downarrow in the small LDL. On diet IV, cholesterol was redistributed to LDL. Walnuts caused HDL to \downarrow , but the apo A-I \uparrow . Apo A-I content of HDL may be regulated primarily by amount of TF in diet, whereas HDL may be more responsive to dietary fat composition. \uparrow PUFA intake \downarrow HDL even in the absence of \downarrow in SFA or MUFA intake. Analyses of plasma fatty acids showed that there was general dietary compliance.	Rationale for sequential vs. random order of diets is based on authors' experience that individuals who are trained to eat a LF diet do not fully resume higher fat intake. 48 g walnuts contain 3.3 g of n-3 FA (α -linolenic acid). Authors showed that 3.3 g of n-3 PUFA found in fish oils \downarrow triacylglycerol by 37%. Absence of BW \uparrow during walnut diet warrants study. Need studies on effects of linoleic and α -linolenic acids on LpL activity. Need to confirm that PUFA-rich walnuts may redistribute plasma lipids from more to less atherogenic LPs. Potential pro-inflammatory and/or carcinogenic effects of n-6 fatty acids warrant further study.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Zambón et al. (81)	M (n = 26), F (n = 23) (mean 56 yr), 22 F were postmenopausal; 6 M and 3 F were hypertensive and on medication; 5 M and 2 F had CHD, all with polygenic hypercholesterolemia. Effects of walnuts on serum lipids and LDL oxidizability in free-living subjects on modified "Mediterranean" diet. Randomized, crossover study.	Patients had LDL >130 mg/dL and triacylglycerol <250 mg/dL, no intake in the previous 8 wk of medications known to affect lipid metabolism; infrequent consumption of nuts and no known allergy to nuts; and no use of multivitamin or vitamin E supplements. 1-wk run-in period during which subjects received dietary advice. Patients came to clinic for a medical visit, interview with dietitian, measurements, and to have blood drawn 2 × in pretrial period and on wk 5 and 6 of each dietary period. Each diet sequence lasted 6 wk, without a washout period. Diets were individually prescribed on estimated energy requirements and calculated in increments of 200 kcal (range from 1,600–2,200 kcal). The control diet was "Mediterranean" (high in MUFA) and was composed of natural foodstuffs. Red meat and eggs were limited, vegetable products and fish were emphasized, olive oil was indicated for cooking, and no nuts were allowed. Patients were given prepackaged raw, shelled walnuts. In the walnut (56 g/d) diet, walnuts contributed ~18% of the TE and 35% of the TF. Blood was drawn after an overnight fast for TC, triacylglycerol, HDL, apo A-1, apo B, VLDL, LDL, and lipoprotein measurements.	Substituting walnuts for part of the MUFA in a cholesterol-lowering "Mediterranean" diet ↓ TC (–4.1%) and LDL (–5.9%) in M and F. Walnut diet ↓ oleic acid (MUFA) by 3.9 mol% and linoleic acid and α-linolenic acid (PUFA) ↑ by 6.6 mol% and 0.34 mol%, respectively. The respective mean changes for mol% of oleic (C18:1n-9), linoleic (C18:2n-6), and α-linolenic acids (C18:3 n-3) were—20%, 14%, and 83%. Similar changes occurred in LDL, phospholipids, and triacylglycerol. Mean TC level ↓ 9.0% (0.65 mmol/L, 25 mg/dL) during the walnut diet and 5.0% (0.36 mmol/L, 14 mg/dL) during the control diet. Mean LDL ↓ 11.2% (0.57 mmol/L, 22 mg/dL) during the control diet. Neither diet affected levels of HDL, VLDL, triacylglycerol, or apo A-1. Apo B levels ↓ after the two diets in parallel with LDL. LDL:HDL ↓ 8% during the walnut diet. The α-tocopherol content of the LDL particles and the lag time of CD formation during copper-induced LDL oxidation were similar during both diets. BW was stable throughout the two study periods. Daily walnut consumption was well-tolerated by most patients. 24/24 patients completed the control diet trial and 25/26 completed the walnut diet trial. General adherence to the diets was determined by measurements of the LDL lipids. Unannounced 24-h diet recalls were performed each wk by telephone during the two dietary periods, for a total of 12 recalls/patient. Authors conclude that substituting walnuts for part of the MUFA in the "Mediterranean" diet ↓ TC and LDL in M and F with hypercholesterolemia.	

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
Berry et al. (83)	26 M, normolipidemic, healthy, Yeshiva (Talmudic College) students, 18–24 y of age. Effects of MUFA and PUFA on plasma lipid concentrations and lipoprotein structure and function. Randomized controlled, crossover study.	Subjects were nonsmoking, and had negligible alcohol intake. About half were born in Israel. Students randomly allocated into two groups on a 24-wk crossover study. During first 4-wk run-in period subjects were given house food. At times they recorded 24-h food intake. Diets in each study group were isocaloric (2,800 kcal/d): 96 g fat (33.5%), 100 g protein (15.5%), 325 g CHO (51%), and ~300 mg cholesterol. About 17% of total calories derived from either MUFA or PUFA. There was a washout period of 4 wk (consisting of house diet). Half of students (group 1) began with a MUFA diet (with almonds) during period 1, then a PUFA diet (with walnuts) during period 2. This order was reversed in other half of students (group 2). During both 12-wk periods students lived in. All their meals and food were provided. Students were given dietary advice. Fasting blood for blood plasma lipid determination was drawn periodically. At end of each period, blood was drawn for lipoprotein structure and function studies.	After period 1 for both diets there was a significant ↓ (11%) in TC; after period 2 this ↓ was greater on the PUFA diet (20%). Triacylglycerol ↓ significantly on both diets in period 1, but after period 2 a slight ↑ was seen after the PUFA diet. There was a significant effect for period (seasonality) but not for diet intervention. There were no significant changes in HDL. LDL ↓ significantly on both diets and over both time periods. LDL ↓ after period 2 on the PUFA diet was significant (31%). A significant seasonality effect was seen. There were no significant differences in lipoprotein composition, structure, or function in response to the two diets. TBARS from LDL ↑ significantly in subjects on the PUFA diet. 26/26 students completed dietary period 1; 22/26 completed period 2. Dietary compliance was monitored by analysis of FA composition in RBC membranes. Although it is established that RBC membrane composition is altered by dietary manipulation of essential fatty acids (eg, linoleic acid), authors suggest that dietary enrichment with MUFA (mainly oleic acid) may also change RBC membrane composition.	Homogeneity of students established. Amount (g) of walnuts, almonds, or other dietary components were not given. Fiber content in diet was not controlled. Only nine students in each group were available for complete blood tests at beginning and end of each period. Effects of seasonality on triacylglycerol between two experimental periods need to be studied. This type of study needs to be tested on F and other populations.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Chisholm et al. (80)	M (n = 21), age 45 ± 6.8 y; BMI 28.4 ± 4.3 kg/m ² ; moderate hypercholesterolemia (polygenic hyperlipidemia), without family history of CHD. Effects of two LF diets on lipid metabolism parameters. Randomized, crossover study with 4-wk period in free-living subjects.	After a 1-wk run-in period on a LF (baseline) diet (fat 30% TE), subjects were randomized into Group 1, who continued on the LF diet, and Group 2, who consumed the walnuts (78 g/d) diet first. During the LF diet all fat came from a variety of foods other than nuts. Each diet period was 4 wk. After the first diet, subjects crossed over to the alternate diet. Diets had range of 1,800 kcal/7,533 kJ-3,000 kcal/12,556 kJ. Walnuts contributed 20% of the TE and 55% of energy from fat in the walnut diet, with the rest of the fat coming from foods chosen by the subjects. On the walnut diet, walnuts supplied 53 g of fat, 30 g of which came from linoleic acid and 5.9 g from α -linolenic acid. Subjects were free-living and received dietary advice. After the initial 4-d diet record during the run-in period, 8-d diet records were collected 2 × d/wk during each experimental period wk.	Linoleic acid (C18:2 n-6) ↑ in all FA fractions and α -linolenic acid (C18:3 n-3) ↑ in triacylglycerol in the walnut group. On the walnut diet, the linoleic acid ↑. Triacylglycerol had significant changes in C16:1 ↓, C18:1 ↓, C18:2n-6 ↑, and C18:3n-3 ↑; phospholipid levels of C16:1 ↓, C18:0 ↓, C18:1 ↑, and C18:2n-6 ↑; and cholesterol ester levels of C16:0 ↓, C18:0 ↓, and C18:2n-6 ↑ compared with the LF diet. TC, LDL, and apo B ↓ below the baseline on both test diets, but the apo B ↓ was significant only on the walnut diet. HDL values were similar on both experimental diets, but were significantly higher than at baseline. Myristic acid (C14:0) content of plasma triacylglycerol on the walnut and LF diets were 2.8 and 3.0 mol%, respectively, despite the relatively similar intakes on both diets. 16/21 subjects provided a full set of dietary records. Dietary compliance was determined by FA measurements. Authors conclude that a diet supplemented with walnuts provide a more favorable lipoprotein profile and may be cardioprotective.	
Abbey et al. (79)	16 normolipidemic M; 41.3 ± 9.4 y; 86.1 ± 2.8 kg BW; TC, 5.15 ± 0.24 mmol/L (199 ± 9.3 mg/dL). Effects of walnuts or almonds on FA composition on plasma lipids. Consecutive, supplemental field study.	Subjects on diet with 36% of energy as fat (92 g/d) for 9 wk. Daily supplement of nuts (50% of TF intake) given against a BD. In first 3-wk period, BD was supplemented with raw peanuts (25 g fat), coconut cubes (11 g fat), and a coconut bar (11 g fat), for 47g TF, with P:M:S to match the Australian RD. During next 3-wk period, BD was supplemented with MUFA-rich raw almonds (84 g/d), equivalent to 46 g fat. During final 3 wk, BD was supplemented with PUFA-rich walnuts (68 g/d), equivalent to 46 g fat. Dietary advice was given. BW was monitored at each clinic visit. Overnight fasting blood was drawn on 3 consecutive d at end of each 3-wk period for plasma lipid measurements.	Compared with RD, there was a significant ↓ in TC after supplementation with almonds or walnuts (7% and 5%, respectively) and in LDL (10% and 9%, respectively). Oleic acid (MUFA) was significantly ↓ and linoleic acid (PUFA) was significantly ↑ in walnut diet. There were no significant differences in HDL or triacylglycerol after any of the dietary-intervention periods. P:M:S for the combined supplement for the RD was 0.4:0.7:1 and 1.8:1:1 for the walnut diet. There were no significant changes in BW throughout study. Dietary compliance was generally confirmed by measurements of fatty acids. Authors suggest that PUFA- and MUFA-rich nuts should be included in diet as a replacement for some of SFA because ↓ in TC and LDL are significant in relation to ↓ in CHD.	Three diet periods were matched for energy intake and major dietary components except fiber, which was significantly ↓ for walnuts than the RD. TF intake was the same in each period, although fat composition was different in each diet because of the different supplements used. Consumption of nuts was quite high (50% of total fat in diet).

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Sabaté et al. (75)	M (15 white, 3 Asian); 21–43 y (mean 30 y); normolipidemic; and normal weight subjects. Effects of walnut intake on serum lipids and BP in free-living, healthy M. Single-blind, randomized controlled, crossover study with two 4-wk periods.	Subjects ate the RD during a 5-d run-in phase. Then followed two consecutive periods of 4 wk on each diet. One group (n = 10) consumed the walnut diet during the first period, followed by the RD diet, while the other group (n = 8) reversed the order. There was no washout period. Study staff performing the measurements and analyses was blinded to the subjects' diet sequence. Subjects received all their meals at the nutrition-research kitchen under supervision. Diets were identical except that the walnut diet substituted three servings of walnuts/d (28 g/serving, or 84 g of walnut/2,500 kcal) for portions of some foods in the RD. The RD was designed according to the National Cholesterol Education Program Step 1 diet. TF accounted for 30%, with equal ratios of SF:MUFA:PUFA. The RD did not contain nuts, nut butters, or nut oils. Walnuts contributed 55%, 14%, and 10%, respectively, of the TF, protein, and fiber of the walnut diet; 20% of the calories derived from walnuts. TC, HDL, LDL, and triacylglycerol levels were measured on fasted blood at the end of each dietary period. FA composition of serum cholesterol was determined for each subject at the end of each diet period. BP was measured 2 × during the run-in period, 1 ×/wk during the first 2 wk of each diet period, and 2 × during the last wk of each diet period.	There was a clear crossover pattern in TC and LDL. Mean TC ↓ during the walnut diet (0.58 mmol/L or 22.4 mg/dL), and was < than the RD, a loss of 12.4%. LDL during the walnut diet was 0.47 mmol/L (or 18.2 mg/dL) < than during the RD, a ↓ of 16.3%. HDL during the walnut diet was 0.06 mmol/L (or 2.3 mg/dL) < than during the RD, a loss of 4.9%. LDL: HDL was 2.5 ± 0.7 during the walnut diet, a loss of 3.7 ± 1.0 during the walnut diet. Triacylglycerol during the walnut diet was 9.5 mg/dL < than during the RD. The effect of walnuts on the lipoprotein risk profile was more favorable than that of the recommended RD. There was general compliance with the protocol as determined by FA measurements. Mean BP values did not change during either dietary period. Authors suggest that replacing a portion of the fat in a cholesterol-lowering diet with walnuts further ↓ serum cholesterol by >10% in normal M. If the effect of walnuts on cholesterol is assumed to be proportional to intake, an ↑ in the current per capita consumption of 4 g/wk to 28g/d/capita would be expected to ↓ TC and LDL by 4% and 6%, respectively.	Long-term effects of walnut consumption and the extension of results to F or other population groups with hypercholesterolemia need to be studied.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
Berry et al. (142)	17 M, normolipidemic, healthy, Yeshiva (Talmudic College) students, 18–24 y. Effects of MUFA-rich diets on plasma LPs. Randomized controlled, crossover study.	Students were nonsmoking, and had negligible alcohol intake. Subjects with endocrine or metabolic disturbances or any chronic disease were excluded. Students were randomly allocated to a crossover study with two 12-wk dietary periods of MUFA vs. CHO-rich diet while SFA and PUFA were kept similar. Half the students (group 1) began with the MUFA diet in period 1 and were given the CHO-rich diet in period 2. This order was reversed in the other half of the students (group 2). There was a 4-wk washout period between each experimental dietary period during which the students were given the regular diet. Students were given dietary advice. MUFA diet had 32.5% energy as fat compared with 18.3% in CHO-rich diet, and had more SFA (4.7%) and PUFA (6.6%) than CHO-rich diet. CHO provided 50.5% of energy in the MUFA diet and 64.9% in the CHO-rich diet. In MUFA diet, fat was added as olive oil, avocado, and almonds. In the CHO-rich diet, part of the fat and MUFA were substituted by CHO-rich food items. Fasting blood for blood plasma lipid determination was drawn periodically. At end of each period, blood was drawn for lipoprotein structure and function studies. Human skin fibroblasts, bovine aortic SMCs, and mouse peritoneal macrophages were grown in vitro. LDL-receptor-specific degradation was determined in monocytes. TBARS determinations were made on SMCs.	TC ↓ significantly by ~7.7% and LDL ↓ by 14.4% on the MUFA diet. There were no significant changes in cholesterol on the CHO-rich diet. HDL did not change on either diet. On the MUFA diet, there was a proneness to peroxidation of plasma and LDL lipids and less metabolism of conditioned LDL by mouse peritoneal macrophages. Blood values were available for seven students throughout the experiment. The initial BW of these students was 68.9 ± 10.8 kg; the BMI (kg/m^2) was 22.4 ± 4.1 . MBW and BMI of the ten subjects in group 2 were 67.3 ± 9.2 kg and 21.4 ± 1.6 , respectively. BW and BMI did not change significantly on MUFA diet during either period and on CHO-rich diet during period 2. However, MBW ↑ by 1.9 kg and BMI by 0.6 in subjects on the CHO-rich diet. On the MUFA diet, the oleic acid content of RBC membranes ↑ significantly during period 2 and the same tendency was observed during period 1. The percentage of PUFA ↓ significantly during both periods. Thus, the oleic acid: α -linolenic acid ratio ↑ significantly. On the CHO-rich diet, the oleic acid: α -linolenic acid ratio did not change significantly. SFA in RBC membranes did not change during both diets. 25/25 students completed the first dietary period; 17/22 completed the second dietary period. Report is on these 17 students. Dietary compliance was monitored by analysis of FA composition in RBC membranes. Authors conclude that dietary MUFA ↓ TC and LDL independently of other dietary fatty acids and may ↓ the susceptibility of LDL to oxidative stress.	Blood values were available for only seven students throughout the experiment. Subjects consumed a relatively high proportion of PUFA in their basic diets, as shown by RBC membrane FA composition. This finding is in line with the high percent of PUFA in the adipose tissue of the Israel population. The cause of the BW ↑ on the CHO-diet is not apparent. Fiber content of almonds in the MUFA diet was not controlled for.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Macfarlane et al. (111)	12 parous F, none pregnant or lactating, who belonged to a low socioeconomic group participated in the walnut study; 115 other F participated in the studies using the other five nuts. Dietary study on the effects of walnuts, almonds, peanuts, hazelnuts, Brazil nuts, and coconuts on Fe absorption from a bread meal. Parallel arm study.	Meals were eaten on consecutive mornings after an overnight fast, and only water was allowed for 4 h thereafter. Nut meal paste was given with two slices (60 g) of the labeled white bread, margarine, whole milk, and a solution of Fe. On one morning the bread was labeled with ^{55}Fe , and on the other with ^{59}Fe . Two wk later, blood samples were drawn after an overnight fast for measurement of ^{55}Fe , ^{59}Fe , Hb, serum Fe, total iron-binding capacity, and serum ferritin. Each subject then drank a solution labeled with ^{59}Fe and ascorbic acid. Only water was permitted for the following 4 h. Blood was obtained 14 d later, and the absorption of the reference dose of Fe was determined from the \uparrow of ^{59}Fe . This provided a measure of each subject's capacity to absorb Fe. Same protocol as for walnut study was followed using the other five nut species.	All six nuts were potent inhibitors of Fe absorption. For walnuts, Fe absorption was inhibited by 5.9% for the bread meal vs 0.7% for the bread and walnut meal. This effect was reversed by ascorbic acid but a lesser amount was needed for walnuts. Walnuts contain $> 1,500$ mg/100 g polyphenols. These are known to inhibit Fe absorption. Walnuts also contain 982 mg/100 g phytates, which may have a role in the inhibitory effect, but this is controversial. Coconuts had significantly more polyphenols and polyphenols. Authors conclude that the bioavailability of Fe would tend to be low in diets in which walnuts were one of the food staples.	Although a high prevalence of Fe deficiency was documented in these F, subjects were not selected on the basis of Fe deficiency. Ages and BWs of subjects not described.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Spiller et al. (117)	M (n = 12), F (n = 33) Californians; 53 ± 10 y; BW 66 ± 13 kg. Dietary effect of almonds on plasma lipids. Randomized controlled, parallel study.	Subjects were excluded who were taking medications known to alter lipids, and for CHD, diabetes, other major diseases, and any known food allergy. All were hypercholesterolemic and hyperlipidemic: TC 6.49 ± 0.78 mmol/L (251 ± 30 mg/dL); LDL 4.29 ± 0.67 mmol/L (166 ± 26 mg/dL); HDL 1.52 ± 0.41 mmol/L (59 ± 16 mg/dL); Triacylglycerol 3.44 ± 1.63 mmol/L (133 ± 63 mg/dL); TC:HDL 2.02 ± .03. Dietary advice given. Three diets (raw nonpareil California almonds, <i>Prunus amygdalus</i>) (100 g/d); California virgin olive oil (48 g/d); and cheddar cheese (85 g/d) and butter (28 g/d) (control diet) were studied over a 4-wk period after a 1-wk baseline period. The olive oil and control diets were supplemented with 21 g/d of rye crackers to approximate the protein and CHO content of the almond diet.	Almond diet induced significant ↓ in TC and LDL relative to the olive oil and control diets while HDL level was preserved. There was no significant change in BW. Subjects' compliance with the protocol and acceptance of the three study diets was assessed by 3-d diet records, 24-h dietary recalls conducted on random d, and verbal reports at group meetings.	
		During the 4-wk study diet period, all subjects consumed a similar BD, which consisted mostly of whole and unrefined foods that were matched for CHO, protein, and TF content. Dietary fiber content was not matched because fiber-rich foods would have confounded findings by their potential to affect lipids. In each group, ~630 calories/d were added to the BD, ~450 calories of which were supplied by almonds (MUFA-rich), olive oil (MUFA-rich) or butter and cheese (SFA-rich) as the primary sources of fat. TF content of each diet was matched, and the study-provided fats comprised the major portion of fat intake.		

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Spiller et al. (143)	M (n = 13), F (n = 13) hypercholesterolemic subjects (56 ± 12.5 y; range 29–81 y). Effect of almonds on plasma cholesterol and lipoprotein. Consecutive supplemental field study.	Baseline diet was modified for all by limiting meat, fatty fish, high-fat milk products, eggs, and SFA. Subjects placed on a LF, low cholesterol, high-fiber diet. Same batch of raw, nonpareil California almonds was used during study. During the 9-wk almond diet period, subjects consumed 100 g/d almonds, 50 g as whole, unblanched, raw almonds and 50 g as ground almonds (34 g MUFA, 12 g PUFA, and 6 g SFA). Almond oil was the only oil allowed for food preparation. Almonds supplied ~37% kcal from fat. Subjects kept 3-d weighed food records during wks 4 and 8 of study. Dietary advice given. Fasting blood was used for plasma cholesterol and lipoprotein measurements.	There was a rapid and sustained ↓ in LDL without changes in HDL. The differences for TC, LDL, and the TC:HDL were highly significant from zero at wks 3, 6, 8, and 9. There were no statistically significant changes for any of the on-diet values for TC or LDL from wk 3–9. VLDL, HDL, and triacylglycerol remained unchanged. Fat increase was due to MUFA (70% of total fatty acids). BW did not change during the study. 19/26 subjects completed the 3-d food records. Authors conclude that the higher MUFA content of almonds, and possibly other nuts high in MUFA, should not preclude their use in hyperlipidemic diets provided this does not lead to ↑ wt gain.	
Jenkins et al. (128)	10 healthy Canadian subjects (9 normolipidemic and 1 with elevated triacylglycerol and low HDL-C). 7 M, 3 F; 33 ± 4 y of age; BMI of 23 ± 1 kg/m ² . Effect of a diet high in vegetables, fruit, and nuts (viz., raw almonds, cashews, and peanuts) on serum lipids. Randomized, crossover design study.	Two diets: Diet 1, a vegetable diet (n = 7) and Diet 2 (n = 3), the control (subjects' HD diet). Subjects on diet A for 2 wk. Control study period either preceded diet 1 or followed it after an interval ≥ 5 mo to minimize the carryover effect. In Diet 1, the main source of fat (25% of TE) derived from raw almonds, cashews, peanuts, and avocado, which were eaten in limited amounts (nuts, 60–120 g/d). The HD (control) provided 29% of the daily fat intake. Subjects were nonsmokers and none consumed alcohol on a regular basis. Three were lacto-ovo-vegetarians. No subjects were taking medications; none had evidence of clinical diabetes or renal or hepatic disease. Serum analyzed for TC, triacylglycerol, HDL-C, LDL-C, apolipoproteins. Short-chain fatty acids were determined. BW measurements were made. Fasting blood samples were collected before the start and at the end of wk 2 of each dietary phase.	LDL ↓ 33%. Lp (a) levels ↓ 24%. TC:HDL-C (21%), LDL:HDL (30%) and apo B:A-1 (23%) ratios ↓ significantly. Subjects ate a mean of 100 g nuts daily. These provided vegetable protein and fiber and have a good dietary FA profile. Vegetable diet provided significant amounts of the antioxidant vitamin A (as β-carotene) and ascorbic acid, although the vitamin E intake was ≤ the RDA. Authors conclude that a dietary change that eliminated animal products and greatly increased consumption of vegetables, fruit, and nuts produced a range of metabolic effects including a marked reduction in lipid risk factors for CVD. The reduction in apo B was related to increased intakes of soluble fiber and vegetable protein. There were no significant changes in BW.	Study was of short duration and small sample size. Walnuts were not part of the diet. Consumption of 84–100 g nuts daily has been associated with 10–20% reduction in serum cholesterol.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Singh et al. (129)	406 M Indian acute MI patient were placed either on vegetarian Diet A (n = 204) or on their physician's prescription Diet B (n = 202). Patients on Diet A were 50.5 ± 9.3 y, those on Diet B were 52.0 ± 8.3 y. Effect of a vegetarian diet on acute MI outcome.	Vegetarian Diet A included fruits, vegetables, and nuts (almonds and walnuts). Physician's diet was a "prudent" diet.	Group A patient had a significant ↓ in mean serum and LDL-C, triacylglycerol, and a nonsignificant decrease in HDL-C. This group also had a significant decrease (35%) in total cardiac endpoints. Their low-energy diet had significantly lower SFAs, cholesterol, caffeine, and salt, and increased fiber, vegetable protein, vitamins C and E, magnesium, potassium, copper, selenium, and chromium in comparison with the "prudent" diet B; it was administered within 72 h of the MI event.	Subjects' adherence to diets was determined by questionnaire. Walnuts were in diet A, but the amount was not specified.
Colquhoun et al. (120)	Randomized, single-blind, 6-wk dietary intervention trial. Australian subjects (M, n = 7; F, n = 7); 25–59 y of age (46.36 ± 10.44 y); BW, 74.8 ± 10.6 kg. Study of effects of macadamia-rich diet on serum lipids and LPs compared to a HC diet. Random controlled crossover study with 4-wk period.	None of the subjects was taking lipid-lowering medication. All had sedentary occupations. Pre-entry Phase: 4-wk dietary intake analyzed. TE derived from fat (average) 37.1 ± 6.2%, calculated from daily food records. PUFA:SFA was 0.4 and the MUFA:SFA was 0.83. HC Phase: 4-wk HC diet had TE derived from fat ↓ to 21.3 ± 2.7% by modifying the pre-entry diet. To maintain a diet isoenergetic to the pre-entry diet and to ↓ TF intake, each subject's diet was individually modified per the American Heart Association III lipid-lowering diet. PUFA:SFA was 0.40 and the MUFA:SFA was 0.86. Macadamia-rich Phase: Diet consisted of a high-fat intake (42.4% TE), of which 20% TE came from macadamia nuts. The amount of nuts consumed was between 50–100 g (~25–50 nuts/d). The nuts were not cooked or heated. PUFA:SFA was 0.35 and the MUFA:SFA was 2.4 on the macadamia diet. Subjects were randomly assigned to each diet for 4 wk; the order was then reversed for a second 4-wk period. There was no washout period. Blood samples were drawn after an overnight fast from each subject 3 × during the pre-phase and 2 × during the final wk of each dietary period. TC, HDL-, and triacylglycerol values were determined.	The macadamia diet led to a 20.9% ↓ in triacylglycerol. HDL ↓ 13.1% with HC diet. Both diets were equally effective in ↓ TC and LDL. The percentage variations in pattern changes in lipids and lipoprotein were similar for the two crossover groups. There was no order or gender effect of the diets on these parameters. 5/14 subjects were nonresponders, with a <5% ↓ in TC on either diet. Dietitian weighed subjects weekly and noted adherence to diets. Authors conclude that a MUFA-rich diet is as effective as a diet high in complex carbohydrates. Macadamia nuts are an effective source of enriching the diet with MUFA.	Macadamia nuts (<i>Macadamia integrifolia</i>) are a native Australian food rich in MUFA (>74% energy), with 58–65% energy derived from oleic acid, and 15–20% energy from palmitoleic acid (16:1 n-7). The cause for the greater ↓ in TC on both test diets is not readily apparent.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Edwards et al. (122)	<p>M (n = 4) (41–53 y of age), F (n = 6) (28–64 y of age), median age 46 y. All patient had moderate hypercholesterolemia; median BW 66.9 kg; median BP 120/78 mm Hg.</p> <p>Effect of pistachio nuts on serum lipids in patient with moderate hypercholesterolemia. Randomized controlled, crossover study with 3-wk period.</p>	<p>Excluded from study were patient, with renal failure, hyperlipidemia, and any on medications. Smokers were not excluded, but no subject smoked during the trial. Although not excluded, no subject was vegetarian.</p> <p>Subjects were asked to maintain lifestyle habits, and they kept diaries to record any signs of illness and to record any new medications taken. Subjects served as their own controls.</p> <p>Half of the patient, were randomized to a pistachio diet for 3 wk and the other half continued on their regular diets for 3 wk. Roasted, unsalted pistachio nuts were substituted for 20% of the daily caloric intake. Patients, otherwise ate the components of their regular diets. For those who ate high fat snacks, pistachio nuts were substituted as fat calories.</p> <p>Patients kept 1-d food records for each of the 6 wk. Lipid profiles were measured during an initial visit (baseline) and on d 5 and 7 of the third wk of each dietary period. Subjects were then crossed over and lipid profiles were measured in the same way. There was no washout period between the two diets.</p> <p>Fasted blood was used for measurements of HDL, LDL, and triacylglycerol values. BW was recorded during the RD and experimental diet.</p>	<p>After 3 wk on the pistachio diet, TC ↓, TC:HDL, and LDL:HDL ↓ significantly, triacylglycerol, LDL and HDL ↓, but not significantly.</p> <p>Pistachio nuts led to a significant ↓ in SFA consumption and an ↑ in MUFA and PUFA when compared with the RD.</p> <p>There were no significant changes in BW and BP during the study.</p> <p>One-d food records kept by patient for each of 6 wk were analyzed to ensure that the appropriate quantities of pistachio nuts were consumed.</p> <p>Authors conclude that a substitution of 20% of daily fat calories with pistachio nuts for other high fat snacks may be beneficial in moderating CHD risk factors by improving lipid profiles.</p>	

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
O'Byrne et al. (126)	36 postmenopausal, hypercholesterolemic F, 50-65 y of age. Effects of LFMR diet containing high-oleic peanuts on serum TC, HDL-C, LDL-C, triacylglycerol, apolipoproteins, and lipoprotein oxidation. Dietary advice, parallel arm.	20 subjects previously consuming high-fat diets (34% energy, SFA, 11%) were placed on LFMR diet (MUFA, 14% energy, LF, 26%) for 6 mo. 16 hypercholesterolemic F already eating LF diets (24% energy) (LF, 20-30%, <10% SFA) were also followed to monitor variations in serum lipids due to seasonality. The LF group participated in cooking and nutrition classes with the LFMR subjects. Dietary advice was provided.	Triacylglycerol and apo A-1 were adversely affected in the LF group. The LFMR diet resulted in significant ↓ in TC and LDL, and a trend toward improved LDL: HDL and apo A-1:apo B ratios. Subjects in the LFMR group ↑ dietary MUFA and ate >2 × the proportion of energy as MUFA compared to the LF group. FA ratios were similar in both groups at baseline and did not change in the LF group. The dietary FA ratios were ↓ in the LFMR group: SFA: MUFA, SFA:UFA, PUFA:MUFA, SFA:fat, and PUFA:fat. The MUFA:fat ↑ significantly so that LFMR subjects were consuming > than half of all dietary fat as MUFA. The LFMR group had a gradual and continuous trend toward wt ↓ during the entire study. As a consequence, their BMI ↓ and body fat was slightly lower. Dietary fiber ↑ was only significant for the LFMR group. The final diets of all subjects provided at least 75% of the RDA for major vitamins and minerals (data not shown). This diet was well-tolerated. 25/36F completed the study (LFMR, n = 12; LF, n = 13). A high-oleic acid peanut cultivar was developed at the University of Florida in which 76-80% of the lipid content is MUFA, ~60-70% oleic acid (more than in most peanut cultivars). Because peanuts are high in arginine, the LFMR diet resulted in significantly ↓ lysine:arginine. The joint classes may have inadvertently influenced the LF group to further restrict %fat, %SFA, %PUFA, and dietary cholesterol. Authors suggest that the use of high-oleic acid peanuts as the primary source of MUFA may be more advantageous than typical high-oleic oils or other vegetable oils.	The proinflammatory and/or carcinogenic potential of n-6 fatty acids warrants study.

TABLE 2 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
A. Human clinical intervention studies				
Curb et al. (119)	M (n = 16) and F (n = 18), age range 18–53 y, weighing between 80–130% of ideal weight, with fasting cholesterol level \geq 3.9 mmol/L (150 mg/dL) and a triacylglycerol level \leq 4.5 mmol/L (400 mg/dL). Effect of macadamia nuts on serum lipid levels. Randomized controlled, crossover trial.	Three diets were studied for 30 d each: a usual “American” diet; a high-MUFA diet (with finely ground macadamia nuts), and an American Heart Association Step 1 “prudent” diet. A 10-d cycle menu used whole foods to match the nutrient profile. There was a 6-d run-in period. For the first dietary period, subjects were randomized to one of three study diets. Then, subjects were randomized to the remaining diets they would follow for the next two periods. All diets had 17% TE from protein. The usual “American” diet had 37% of energy from fat (16% SFA, 14% MUFA, and 7% PUFA). The American Heart Association Step 1 diet had 30% energy from fat (9% SFA, 14% MUFA, and 7% PUFA). The macadamia nut diet had 37% of energy from fat (9% SFA, 21% MUFA, and 7% PUFA). The cholesterol content (300 mg) of all diets were kept constant. Up to 5 alcoholic beverages/wk and 5/d non-energy-containing beverages with caffeine were allowed. Daily breakfast, dinner, and a bag lunch were provided. Subjects were allowed a “free” meal on Saturday night with specific guidelines on the amount of fat consumed. Subjects were stratified by sex. BW measured 2 \times /wk. Fasting blood drawn on the last 3 consecutive d of each dietary period for analyses of TC, HDL-C, and triacylglycerol levels. LDL-C levels were determined. Study personnel involved in performing measurements and analyses were blinded to the diet sequences. Subjects were not taking medication for hyperlipidemia; had no history of diabetes or pancreatic insufficiency; had no history of food allergies; were not pregnant, breast feeding, or taking birth control pills. BW was measured 2 \times /wk.	Analyses: Compared with the usual “American” diet (TC level: 5.20 mmol/L, 201 mg/dL), the mean TC level was significantly lower for the macadamia nuts (4.95 mmol/L, 191 mg/dL) and American Heart Association Step 1 (4.99 mmol/L, 193 mg/dL) diets. LDL-C Analyses: The mean LDL-C level was also lower on the two experimental diets (3.22 mmol/L, 125 mg/dL on the macadamia nut diet and 3.21 mmol/L, 124 mg/dL on the American Heart Association Step 1 diet), whereas it was 3.37 mmol/L, 130 mg/dL on the usual “American” diet. TG Analyses: Mean TG levels were significantly higher than with the usual “American” diet (0.87 mmol/L, 77.5 mg/dL) for the American Heart Association Step 1 diet (0.94 mmol/L, 83.6 mg/dL) and significantly lower for the macadamia nut diet (0.79 mmol/L, 70.4 mg/dL). HDL-C Analyses: The mean HDL-C level was lower (by about 4%) after the American Heart Association Step 1 and the macadamia nuts diets. Lipid profile trends on the modified diets were not statistically different for M and F. The authors suggest that replacing SF in the usual “American” diet with MUFA present in macadamia nuts has a favorable effect on serum cholesterol levels of healthy adults. They emphasize replacement of some SFA (and not supplementation) because of the adverse effects associated with weight gain. Authors recommend a 30-d diet period as the minimum that should be used to study dietary effects on lipids. Subjects' compliance with regimens were determined by telephone screening, individual and group meetings, and from the 6-d run-in period.	Macadamia nuts contain 19.29% (20.3% w/w) of palmitoleic acid (16:1 n-7). This MUFA has not been much studied (compared to other fatty acids), so that any benefits or adverse effects conferred by it are not known. The daily intake of macadamia nuts was not specified.

TABLE 2 (continued)

B. Animal intervention studies	Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
	Ravel et al. (132)	Study of growth rate and hypocholesterolemic effect of walnut meal compared to soybean meal on New Zealand rabbits. 6-wk laboratory animal study.	Five groups (I-V) of 8 F New Zealand rabbits each, 8-10 wk of age, BW 2,368 ± 75 g, were housed in cages to avoid coprophagy during the 6-wk study of different diets. Group V rabbits were fed a semisynthetic diet based on the defatted soy diet with 25% defatted walnut meal replacement. Necropsy was performed at wk 7. Liver, spleen, kidneys, adrenals, and aorta were weighed and sections made for histopathology. TC and lipoprotein levels were made from frozen and lyophilized specimens. Amino acid composition was determined. Growth curves were made using average BW/wk. Measurements were made at start of trial (t ₀), at wk 3 (t ₁), and end of trial (t ₂), of TC, triacylglycerol, total Ls, urea, and proteins.	The walnut diet ↓ cholesterolemia to 1.63 g/l. Hypertriglyceridemia is correlated with hypercholesterolemia. Authors suggest further study on its cause to see if it is related to VLDL metabolism or apoproteins. Liver and adrenal wt ↑ on the walnut diet compared to the soy diet. 6/8 animals survived to the end of the trial. Survivors had BW 2601 ± 82 g, with a net ↑ of 233 g, for a 10% ↑ over the initial BW. The hypocholesterolemic factor in walnut diet remains to be elucidated. It may be due to the amino acid mix, which is methionine-poor and to the lysine:arginine.	Loss (25%) of animals is not explained; one was lost to study at wk 1 and the other at wk 6. The underlying mechanism for the increase in organ weight on the walnut diet is not yet clear.
	Ravel et al. (133)	Effect of dietary proteins on platelet aggregation, blood coagulation, and plasma lipids in F New Zealand white rabbits. 6-wk laboratory animal study.	6-wk study using eight animals/diet. The three semisynthetic diets were walnuts, soybean, or casein. Walnut and soybean meals (oil cakes) were defatted by hexane extraction. BW at start of trial ranged from 1,860-1,960 kg. Each rabbit was given 150 g/d of diet and ad libitum water. TC, HDL, phospholipid, and triacylglycerol values were determined. During the first 5 wk, animals were fed diet containing 4% mixed fats. At end of this period (t ₀), blood was drawn to establish baseline values of serum triacylglycerol, TF, phospholipid, and TC. These parameters were also measured on blood samples drawn at wk 6 (t ₁) and wk 12 (t ₂) of the experimental period. Animals were divided into two groups (n = 10 each) and fed during 12-wk experimental period either diet I (containing 10% corn oil) or diet II (containing 10% walnut oil). Both semisynthetic experimental diets contained 0.1% cholesterol, 2.5% casein, 39.9% sucrose, 5% minerals, 1% vitamins, and 19% cellulose.	Addition of walnuts to diet ↓ TC and platelet aggregability. The relative effects of the three dietary proteins best correlated with the lysine:arginine in the protein and the HDL:LDL. Platelet fatty acids had ↑ in C20:5 n-3/C20:4 n-6 in walnut- and soybean protein-fed animals, but the changes were greater in the latter while inhibition of platelet aggregation was greater in walnut protein-fed animals. No significant difference was observed among the three diets in coagulation tests. Diets I and II were hypercholesterolemic but did not ↓ TC, phospholipid, or TF. Walnut oil ↓ platelet aggregation more than corn oil. Authors conclude that platelet function is not correlated with a hypocholesterolemic effect of a dietary fat and persists when the rabbits are fed hypercholesterolemic diets.	Ages of animals not characterized.
	Roussel et al. (134)	20 New Zealand rabbits, 2.5-2.1 kg. Dietary study of the effects of polyunsaturated oils (walnut and corn) on lipid metabolism and platelet aggregation. 12-wk laboratory animal study.			Ages and sex of rabbits not described.

TABLE 3
Observational studies

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
Fraser (85)	AHS, non-Hispanic, white M and F SDAs in California. 34,000 subjects enrolled. Associations between nut consumption and risk of CHD. Prospective 12-y longitudinal cohort study.	Survey of published studies on different diets and cohort nutritional populations, with a consideration of nut consumption, lipids, and risk of CHD.	The frequent eating of nuts, including walnuts, is associated with a 30 to 50% decreased risk of CHD.	
Fraser (91)	AHS, California non-Hispanic, white M and F SDAs. 34,192 subjects ≥ 25 y of age at study baseline (1976). Comparison of different dietary patterns among SDAs and risk of cancer, IHD, and other diseases. Prospective 12-y longitudinal cohort study.	Baseline data collected from responses to mailed census (1974) and lifestyle (1976) questionnaires. FFQ on 51 different foods or food groups with eight frequency categories, ranging from never to $>1 \times/d$. Three categories of dietary habits among SDAs are vegetarian, those who ate no fish, poultry, or meat (29.5%); semivegetarian, those who ate fish and poultry, but $\leq 1 \times/wk$ (21.2%); and nonvegetarian (49.2%). Only 2–3% of SDAs are vegans. A surveillance program to detect new cancer and IHD cases was conducted, consisting of annual mailings to every member of the cohort. Computerized record linkage for tumor registries in Los Angeles County and the San Francisco Bay Area. Deaths were identified through state death tapes and the National Death Index. International Classification of Diseases criteria were used to confirm diagnoses of nonfatal MI. Multivariate analyses were used to show associations between dietary patterns and fatal IHD in M, significant protective associations between nut consumption and fatal and nonfatal IHD in M and F, and reduced risk of IHD in subjects preferring whole grain to white bread. Survey of three cohorts of California AHS/SDA dietary patterns, and the epidemiology and risk of CHD in F.	The food that was most consistently associated with reduced risk of both fatal and nonfatal IHD was nuts. Subjects who ate nuts 4 to 5 \times/wk had only $\approx 50\%$ of the risk of those who ate nuts $\leq 1 \times/wk$. The association was independent of confounding with vegetarian status. Other traditional risks of IHD (ie, diabetes, hypertension, past smoking, obesity, and physical inactivity) were seen in this cohort as in other study populations.	The particular types of nuts consumed were not specified.
Fraser (144)	AHS, California, non-Hispanic, white, postmenopausal F SDAs (average age, 53 y). Review of published studies on three AHS/SDA, California cohort groups on diet as primary prevention of CHD.		About 25% of the SDA subjects consumed nuts 4 to 5 \times/wk (compared to only 5% of the American population). Fatty food, specifically nuts, are protective for CHD, especially in F in these cohorts.	

TABLE 3 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
Fraser & Shavlik (90)	AHS, California non-Hispanic white M and F SDAs. 603 subjects ≥ 85 y at study baseline: 178 M (85–99 y) and 425 F (85–100 y). This subgroup is called the oldest-old population. Prospective 12-y longitudinal cohort study.	Baseline data collected from responses to mailed census (1974) and lifestyle (1976) questionnaires. Proportional hazards survival analyses used attained age as the time variable and included all subjects. The proportional hazard assumption is that the RR associated with the exposures is constant from 85 y until the end of the lifespan.	There were 364 cases of CHD and 1,387 total deaths. M had $>$ risk (36%) of both all-cause mortality and mortality from CHD. The sex ratio in this population is similar to that reported from the U. S. Bureau of the Census, at 41.9 M/100 F aged ≥ 85 y. Subjects who ate nuts $5 \times$ /wk had RR of death of 0.82 and 0.61 for death from CHD compared with those consuming nuts $< 1 \times$ /wk. Authors conclude that even in the oldest-old, certain traditional risk factors and dietary habits are associated with mortality.	
Fraser et al. (89)	AHS, California African American M (n = 585, 52.0 ± 15.4 y) and F (n = 1083, 53.4 ± 15.2 y) SDAs. Study of association among health habits, risk factors, and all-cause mortality. Prospective 11-y longitudinal cohort study.	Baseline data were collected from responses to mailed census questionnaire (1974) from the AHS household residents, irrespective of religion, with at least one SDA. Responses were returned by 3,299 respondents. A lifestyle questionnaire was mailed (1976) to the 3,537 African American subjects whose heads of household responded to the census questionnaire. This elicited a response from 1,740 (49.2%) subjects. Mortality data follow-up continued through 1985. Exposure information was collected from both questionnaires. The cohort status was ascertained using church records and the California State death records. Vital status was determined for 93% of the subjects; the remaining 7% were considered lost to the study. A 65-item FFQ (1976) asked about dietary use including "nuts (except those used in recipes)." Multivariate proportional hazard survival analyses were used to evaluate hazard risk (both sexes combined) for exposure variables on all-cause mortality and to adjust for cause mortality and to adjust for potentially confounding variables.	Authors conclude that traditional risk factors operated with similar force in this African American cohort. Authors conclude that the frequent consumption of nuts ($\geq 5 \times$ /wk) was associated with lower hazard risk (0.6 for M, 0.5 for F, 0.6 for both sexes) for all-cause mortality. The lower hazard risk was confirmed when controlling for vegetarianism.	Authors reported the frequency of consumption of foods separately for M and F, but did not specify the types of nuts eaten or the serving quantities. Since "nuts used in recipes" were excluded by the FFQ, it is likely that the respondents underestimated their nut consumption.

TABLE 3 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
Fraser et al. (88)	AHS, 27,321 California non-Hispanic white M and F SDAs, >24 y in 1974, with no known CHD in 1976. Prospective 12-y longitudinal cohort study.	Baseline data collected from responses to mailed census (1974) and lifestyle (1976) questionnaires. Effect of traditional CHD risk factors on lifetime risk, age at onset, and survival free of CHD, and two dietary variables (viz., nut consumption and vegetarianism) that showed significant associations with CHD risk. Outcome measures were estimated with the use of multiple decrement life tables: 1) lifetime risk; 2) mean age at onset (fatal or nonfatal CHD combined); 3) life expectancy free of CHD, which allowed either onset of the disease (fatal or nonfatal combined) or death from a competing cause as part of a composite endpoint.	Frequent nut consumption ($\geq 5 \times/\text{wk}$) results, on average, in lifetime risk of CHD being ↓ by 12.4 percentage points.	
Fraser et al. (87)	AHS, 26,473 California non-Hispanic white M and F SDAs. M (n = 10003) (51.3 ± 16.0 y) and F (n = 16740) (53.2 ± 16.6 y) with no known CHD. Study of consistency of the association between nut consumption and risk of CHD. Prospective 12-y longitudinal cohort study.	Baseline data collected from responses to mailed census (1974) and lifestyle (1976) questionnaires. Extensive dietary information was obtained along with values of traditional confounding CHD risk factors (viz., age, BMI, hypertension, smoking, exercise, and education). These were related to risk of definite fatal CHD or definite nonfatal MI. Age- and sex-stratified analyses within a number of the 16 population subgroups were used to explore the association between nut consumption and risk of CHD.	34% of the subjects ate nuts <1 \times/wk , but 24% ate nuts $\geq 5 \times/\text{wk}$. Consistent strong negative associations between consumption of nuts and risk of CHD were significant statistically. Authors conclude that there is an inverse relationship between W dietary consumption and frequency of consumption and the risk of CHD.	Potential limitations of study design include possible selection bias, misclassification of exposure, and confounding. However, the reported associations persist.

TABLE 3 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
Hu et al. (27)	NHS, 86,016 F RNs, 34–59 y of age without previously diagnosed CHD, stroke or cancer in 1980. Study the relationship between peanut and other nut consumption and risk of CHD including non-fatal MI and fatal CHD. 14-y longitudinal prospective cohort study.	NHS baseline data (1980) collected on subjects' medical history and lifestyle and updated from questionnaires mailed every 2 y. In 1980, dietary questionnaire comprised 61 items. It was expanded to 116 items (1984), and updated in 1986 and 1990. In 1980 and 1984 subjects were asked how many times, on average, during the previous y they had eaten nuts [1 unit equivalent to 1 oz (28 g) of nuts]. Responses for nuts ranged from almost never to $\geq 5 \times$ /wk. Responses were based on frequency distribution of the variables. In 1986 and 1990, the question was divided into two, one for peanuts and other one, nuts. Consumption of total nuts in both 1986 and 1990 was the sum of the two items combined. The correlation coefficient between intake of nuts assessed by the 1980 questionnaire and by 4-wk dietary records was 0.66.	Nut consumption in the cohort \downarrow during the 1980s. In 1980, $\sim 9\%$ of subjects reported eating one unit of nuts 2–4 \times /wk, and 5% $\geq 5 \times$ /wk. By 1990, the corresponding percentages \downarrow to 4.5% and 3%, respectively. 1,255 major CHD events (861 cases of nonfatal MI and 394 cases of fatal CHD) occurred during the 14-y follow-up. After adjusting for traditional risk factors for CHD, F who ate > 5 units of nuts/wk had a significantly \downarrow risk of total CHD (RR 0.65) ($\sim 35\%$) than F who ate < 1 unit/mo. The magnitude of risk \downarrow was similar for both fatal CHD and nonfatal MI. Further adjustments for intakes of dietary fats, fibers, vegetables, and fruit did not alter these results. The inverse association persisted in subgroups stratified by levels of smoking, use of alcohol, use of multivitamin and vitamin E supplements, BMI, exercise, and intake of vegetables or fruit.	Peanuts are identified, but "nuts" are not further characterized by specific type. Possibility of residual confounding cannot be ruled out, but it is unlikely that it can fully explain the observed strong inverse association between frequent nut consumption in these subjects and \downarrow risk of CHD.
Kushi et al. (92)	Iowa Women's Health Study, 34,486 post-menopausal F, ages 55–69 y, with no CHD (1986). Effects of dietary intake of antioxidants and relationship to fatal CHD. 7-y prospective study.	Baseline data collected from FFQ similar to NHS (1984) FFQ, augmented by questions on type of fat used, brand of cooking oil, names of other regularly consumed foods, the brand names of multivitamin preparations and breakfast cereals; current use and dosage of supplements of specific vitamins. F excluded if the FFQ had > 29 items left blank; if their reported energy intake was implausibly low or high, or if they had angina or CHD or a heart attack. To evaluate the reliability of the questionnaire, the vitamin intake in a subgroup of 44 F with their mean intake was estimated from 5 24-h dietary-recall interviews.	Analysts adjusted for age and dietary energy intake, vitamin E intake with the risk of CHD. This association was marked in the subgroup of 21,809 F who did not consume vitamin supplements. After adjustment for possible confounders, this inverse relationship remained. There was little evidence that the intake of vitamin E from supplements was associated with a \downarrow risk of fatal CHD. The effects of high-dose supplementation and the duration of supplement use could not be addressed. Intake of vitamins A and C did not appear to be associated with the risk of fatal CHD. Multivariate adjusted RR (95% CI), also adjusted for vitamin E intake from consumed nuts, of death from CHD among 19411 postmenopausal F who did not take vitamin supplements supports an inverse association of vitamin E intake with fatal CHD. Nuts are among the most concentrated food sources of vitamin E.	

TABLE 3 (continued)

Reference	Subject and description	Methods and outcome variables	Results and authors' conclusions	Comments
Lavedrine et al. (109)	793 subjects M (n = 426) 48.57 ± 12.55 y; F (n = 367) 50.73 ± 11.25 y) of a population of small family farmers from villages in a walnut production area (Dauphiné) near Grenoble, France. Cross-sectional survey.	Data collected (1994) on FFQ on past diet (1-y recall including walnut and animal fat consumption) and CHD risk factors. Excluded were persons who were hyperlipidemic or hypotensive and under treatment. HDL, LDL, TC, apo A-I, and apo B were determined on each subject from blood sample. Multiple linear regression models were used in which the expected (average) value of each level of serum lipid (HDL and apo A-1) was modeled with gender, age (18–45, 46–55, 56–65 y), BMI, alcohol, animal fat, and walnut and/or walnut oil consumption. Separate models were tested for each serum lipid. Nutritional fat categories were defined by three categories on the basis of the authors' knowledge of the typical nutritional habits of the farmer population in their region: poor, high, and moderate animal fat consumers. Based on frequency, there were three categories of users of walnut oil: nonconsumers, frequent consumers (those who use walnut oil every d), and intermediate consumers (all others). Smokers were noted. For alcohol consumption, subjects were grouped into moderate drinkers (<40 g/d) and heavy drinkers (at least 40 g/d).	High levels of HDL and apo A-1 were associated with a high dietary intake of walnuts (oil and kernel), and a positive trend with ↑ walnut consumption. Dietary animal fat and alcohol did not appear to be confounders. Other blood lipids did not show significant associations with walnut consumption. Very few subjects were smokers (8.2%). All subjects consumed alcohol. There were very few subjects who were walnut-only consumers. Authors conclude that walnut consumption has a positive association with blood HDL and apo A-1 and an inverse association with CHD.	Study is not quantitative since it depended on 1-y recall on FFQ. This dietary study is different from others because it evaluated a general population eating its usual diet. It would be useful to determine whether these results hold true for other walnut types, according to their FA content and amino acid composition.

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