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Dietary Fat and Risk of Cardiovascular Disease: Recent Controversies and Advances

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diet, fat, cardiovascular disease, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids

Abstract

Health effects of dietary fats have been extensively studied for decades. However, controversies exist on the effects of various types of fatty acids, especially saturated fatty acid (SFA), on cardiovascular disease (CVD). Current evidence supports that different types of dietary fatty acids have divergent effects on CVD risk, and the effects also depend strongly on the comparison or replacement macronutrient. A significant reduction in CVD risk can be achieved if SFAs are replaced by unsaturated fats, especially polyunsaturated fatty acids. Intake of industrially produced *trans* fat is consistently associated with higher CVD risk. Both n-6 and n-3 polyunsaturated fatty acids are associated with lower CVD risk, although the effects of fish oil supplementation remains inconsistent. The 2015–2020 Dietary Guidelines for Americans place greater emphasis on types of dietary fat than total amount of dietary fat and recommend replacing SFAs with unsaturated fats, especially polyunsaturated fatty acids for CVD prevention.

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INTRODUCTION

For decades, the effects of various dietary fatty acids on the risk of cardiovascular disease (CVD) has been extensively studied through multiple approaches, including in vitro studies, animal experiments, controlled feeding studies, observational epidemiologic studies, and randomized controlled trials (RCTs). Interest in dietary fats and CVD emerged as early as the 1930s from an observation that dietary cholesterol contributed to the development of atherosclerosis by elevating serum cholesterol in animal experiments (39). In the 1940s and 1950s, controlled feeding studies and the Seven Countries Study by Ancel Keys (69) collectively suggested a major role for the type and amount of dietary fat in determining serum cholesterol. In 1961, the American Heart Association recommended replacing saturated fatty acid (SFA) with unsaturated fatty acids to prevent CVD (11). However, in the 1980s and 1990s, these recommendations shifted to an oversimplified low-fat message, i.e., reducing all types of fat and replacing them with carbohydrates. The low-fat campaign led to a substantial reduction in percentage of calories from total fat but a compensatory increase in consumption of carbohydrates, especially refined starch and added sugar. In the meantime, the prevalence of adult obesity soared from 10.0% in the early 1980s to 37.7% in 2014 (32, 33), the incidence of type 2 diabetes doubled (11), and the decades-long decrease in CVD plateaued (98). Since the beginning of this century, various dietary recommendations have begun to recognize the unwanted consequences of the low-fat campaign and reemphasize the role of specific types of dietary fat. Most recently, the 2015–2020 Dietary Guidelines for Americans essentially eliminate the upper limit on total fat intake but retain the recommendations of <10% of calories from SFA and replacing SFA with unsaturated fatty acids (136).

One of the main links between different types of dietary fatty acid and CVD is their divergent effects on serum lipid profiles that are well established by numerous controlled feeding studies (93). Dietary fats may also affect CVD risk via other mechanisms such as endothelial function, cardiac function, chronic inflammation, and blood clotting tendency (146). Further, as indicated in early controlled feeding studies, the effect of a fatty acid can be validly examined only when it is isocalorically exchanged by an explicitly specified macronutrient such as another fatty acid or carbohydrate. The principle of isocaloric comparison has been extended to other study designs, including prospective cohort studies and RCTs of clinical CVD endpoints, and is a key consideration for interpretation of any evidence regarding the health effects of dietary macronutrients (149).

Several recent publications (16, 119, 120) have challenged current dietary recommendations on dietary fats especially the role of SFAs in the development of CVD. These studies and the media attention they attracted have caused widespread confusion in the biomedical community

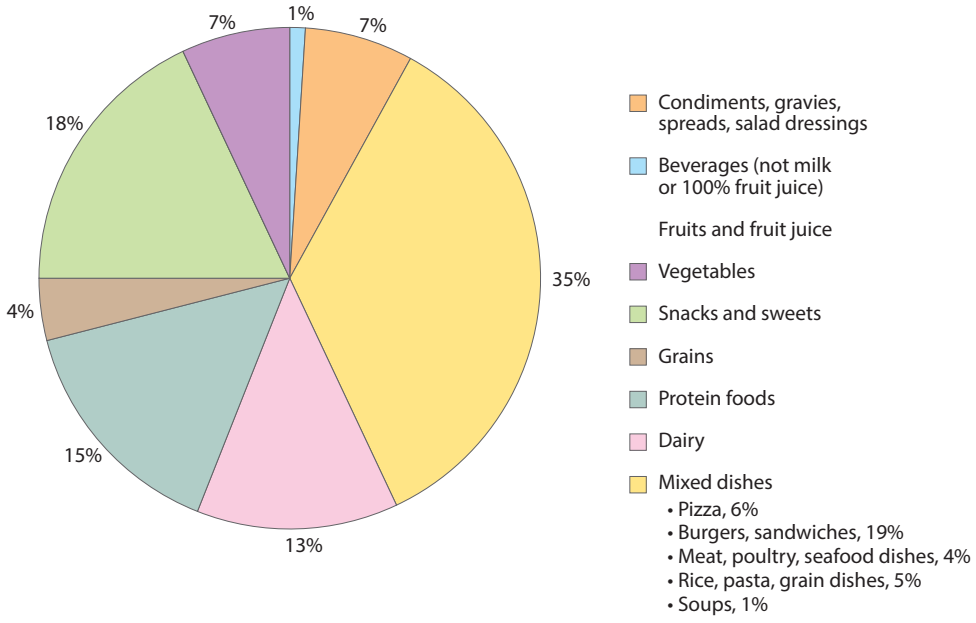


Figure 1

Top food sources of saturated fat among US populations, What We Eat in America, National Health and Nutrition Examination Survey 2009–2010 (26).

and the general public about the importance of types of dietary fat for prevention of CVD. In this review we summarize current evidence on the relation of dietary fatty acids to CVD risk and review some recent controversies and advances in the field.

SATURATED FATTY ACIDS

In the United States, the median intake of SFA was 11.1% of daily energy intake (26); the top food sources of SFA were mixed dishes, particularly burgers and sandwiches, and snacks and sweets (**Figure 1**). Controlled feeding trials in humans have established the effects of dietary SFA on lipid fractions and demonstrated that its effects varied depending on the comparison macronutrients (**Figure 2**) (93). SFAs, when substituted for carbohydrates, increase both low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol and thus have no significant effects on more global lipid makers that predict CVD risk, such as the total cholesterol (TC) to HDL ratio or ApoB levels (93). The quality of carbohydrates, usually rated by the glycemic index (GI), also affects the substituting effects of SFAs on lipid profile because the consumption of lower-GI foods, e.g., whole grains, when compared with higher-GI foods, e.g., refined starch and sugars, has been associated with lower LDL cholesterol and higher HDL cholesterol (81). In contrast, replacement of SFAs with polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) decreases TC and LDL cholesterol and only slightly decreases HDL cholesterol; the TC to HDL ratio is also decreased (93). Compared with its effects on blood lipid, the effects of SFA intake on other CVD risk factors, including systematic inflammation, blood pressure, and insulin resistance, were less well characterized. Except for some suggestion of increased fibrinogen (4) and tumor necrosis factor-α levels (43), several small short-term RCTs that investigated high-SFA

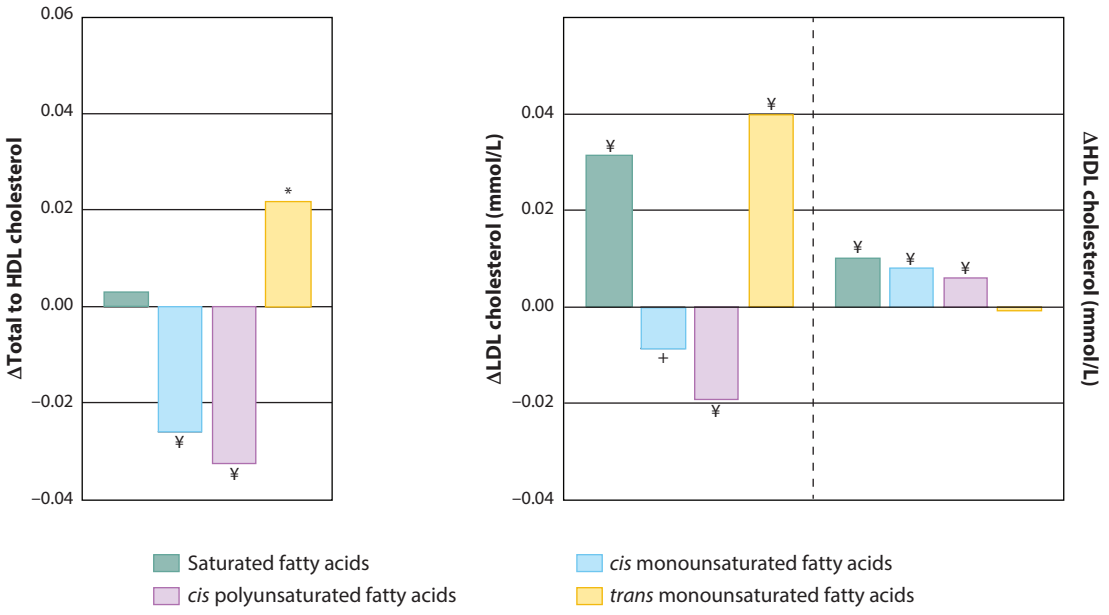


Figure 2

Effects of isocalorically replacing 1% of total energy intake from carbohydrates by the same energy from dietary fatty acids on the ratio of serum total to HDL cholesterol and concentrations of LDL and HDL cholesterol: a meta-analysis of 60 controlled trials. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein. Adapted from Reference 93.

diets generally found no substantial effects on markers of systematic inflammation, such as tumor necrosis factor-α, interleukin-6 and monocyte chemoattractant protein-1, when compared with diets high in MUFA, PUFA, or carbohydrates (4, 43, 66). For blood pressure or vascular function, limited studies generally reported no associations between SFA intake and incident hypertension (2, 42, 153) or arterial stiffness (68, 126). Although SFA consumption in place of MUFA appeared to worsen glucose-insulin homeostasis in several RCTs (114, 139), most prospective cohort studies found that replacing SFAs with carbohydrates was not significantly associated with risk of type 2 diabetes (44, 60, 125, 137).

In most prospective cohort studies, SFA intake was weakly and nonsignificantly associated with coronary heart disease (CHD) risk (16, 59, 109, 130). In a meta-analysis conducted by Siri-Tarino et al., the summary risk ratio (RR) comparing extreme categories of SFA intake was 1.07 [95% confidence interval (CI), 0.96–1.19] (130). In well-conducted studies explicitly comparing dietary SFA with total carbohydrate (59, 84, 109), this weak association was expected and consistent with the lack of effect of substituting SFA with carbohydrate on the TC to HDL ratio. A recent controversy emerged from a meta-analysis conducted by Chowdhury et al., who concluded that their findings did not clearly support current recommendations of replacing SFA with PUFA (16). However, this meta-analysis had several methodological problems. In particular, most studies included in this meta-analysis were not explicit about the comparison nutrient to SFA (152). With the replacement nutrient unspecified, these studies by default compared SFA with all the other sources of calories, which in a typical Western diet are mainly refined starch, sugar, and partially hydrogenated oils. Not surprisingly given this comparison, SFA has been minimally associated with CHD risk. In contrast, prospective cohort studies that evaluated the effect of replacing SFA with PUFA or low-GI carbohydrate found significant cardiovascular benefits (59, 64, 84). For

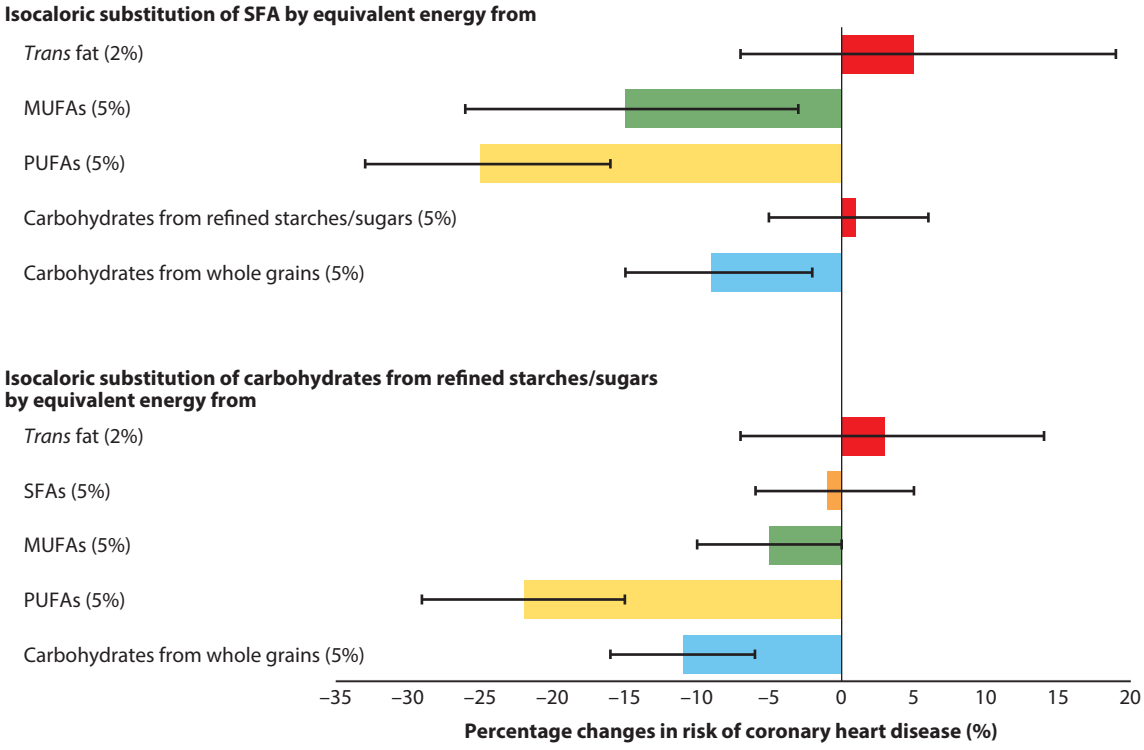


Figure 3

Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. Adapted from Reference 84.

example, in our recent analysis in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) (84), we estimated that replacing 5% of calories from SFA with the same calories from PUFA, MUFA, or carbohydrate from whole grains was significantly associated with 25%, 15%, and 9% reduction in CHD risk, respectively (**Figure 3**).

Although randomized clinical trials (RCTs) can provide a direct test of whether a reduction in SFA intake reduces CHD rate, most dietary intervention studies have been limited by methodological issues, such as poor adherence, high attrition, short duration, and small sample sizes. Different macronutrient replacements for SFA are also crucial for interpreting the RCTs. Very few RCTs have directly tested the effect on CHD risk of replacing SFA with carbohydrate. In the Women's Health Initiative, the low-fat intervention reduced SFA intake by 3% of calories and replaced SFA largely with carbohydrates. However, compared with the control diet, the low-fat intervention did not significantly reduce incident CHD (RR = 0.93; 95% CI, 0.83–1.05) and total CVD (RR = 0.96; 95% CI, 0.89–1.03) during the 8-year follow-up (57). This trial provided strong evidence that simply reducing total fat and SFA is not effective in reducing CVD risk.

Studies that investigated the association between SFA intake and incidence of stroke generally yielded nonsignificant results. In a meta-analysis of eight prospective cohort studies, SFA consumption was not associated with total stroke (130); a recent meta-analysis based on 12 cohort studies, without specifying the comparison nutrient, concluded with an RR of 1.02 (95% CI, 0.90–1.15) for ischemic stroke comparing the highest with the lowest consumption level of SFA (24).

In the Women's Health Initiative, replacement of SFA largely with carbohydrates did not yield a significant reduction in the incidence of stroke (RR = 1.02; 95% CI, 0.90–1.17) (57).

SFAs from different food sources and CHD risk were investigated in the Multi-Ethnic Study of Atherosclerosis and the European Prospective Investigation into Cancer and Nutrition–Netherlands cohort (22, 117). Both studies reported that higher SFA from dairy was associated with lower risk of CHD, whereas null associations were observed for SFA from other sources. On the basis of these findings, some researchers proposed that the effects of SFA may be modulated by their food sources and that SFA from dairy foods may have cardiovascular benefits. However, dairy SFA is highly correlated with other components in dairy such as calcium, magnesium, or potassium; therefore, it is nearly impossible to tease out the effects of SFA in dairy from other dairy components. Our recent study in three prospective cohorts, the NHS, NHS II, and HPFS, found that dairy fat intake, compared with total carbohydrate intake, was not associated with risk of total CVD, CHD, or stroke. By contrast, replacing dairy fat with vegetable sources of fats and PUFAs was related to lower CVD risk (12). In addition, evidence from a long-term RCT supported the beneficial effects of replacing dairy fat with vegetable oils. The Finnish Mental Hospital Study almost eliminated dairy SFA from the intervention diet and substituted it with PUFA: The study replaced whole milk with an emulsion of soybean oil in skim milk and replaced butter and ordinary margarine with soft margarine high in PUFA (135). Compared with the control diet, the intervention group had a significant reduction in both plasma cholesterol and CHD rate. Between 1972 and 2012, the Finnish population experienced a significant reduction in serum cholesterol primarily due to dietary changes: Whole milk was replaced with reduced fat or skim milk, and butter with vegetable oil (67). In parallel with changes in serum cholesterol and other CVD risk factors, there was an 82% reduction in CHD mortality in the Finnish population. Although interesting from a scientific point of view, studies examining SFA from one food source without considering other components in the same source run the risk of providing "off-target" dietary recommendations. Instead of focusing on SFA from different food sources, studies on SFA-rich foods are likely to contribute more useful evidence for actionable dietary guidelines.

The effects of SFA on blood lipids vary depending on the carbon-chain length of individual SFAs. Stearic acid (18:0) has no effect on the TC to HDL ratio, whereas shorter-chain SFAs, such as lauric (12:0), myristic (14:0), and palmitic (16:0) acids, show greater LDL cholesterol-raising effects. An increase in lauric acid intake also leads to a substantial increase in HDL cholesterol and a significant decrease in the TC to HDL ratio (93). Several studies have attempted to distinguish potentially divergent associations of individual SFA with CHD. Although there was a suggestion that longer-chain SFAs such as palmitic acid, the most abundant SFA in a typical Western diet, was associated with stronger risk of CHD versus other SFAs, it is not practical to distinguish individual SFAs with different carbon-chain lengths in dietary recommendations because there are very high correlations among these SFAs given shared food sources such as dairy and meats (58, 160).

n-6 POLYUNSATURATED FATTY ACIDS

n-6 PUFAs, characterized by the presence of at least two double bonds with the first double bond at the sixth carbon from the methyl terminus, are the main PUFAs found in vegetable oils, nuts, and seeds. The major dietary n-6 PUFAs include linoleic acid (LA) and arachidonic acid (AA): Whereas LA is the predominant dietary PUFA, AA has a very low consumption level. Controlled feeding studies have consistently shown that replacing either SFA or carbohydrate with LA reduces LDL cholesterol and TC to HDL ratio (73, 93). However, some investigators have long advocated that excessive LA intake is a culprit of risk of inflammatory disease like CHD, citing several earlier experimental observations that AA, an LA metabolite, could be converted to proinflammatory

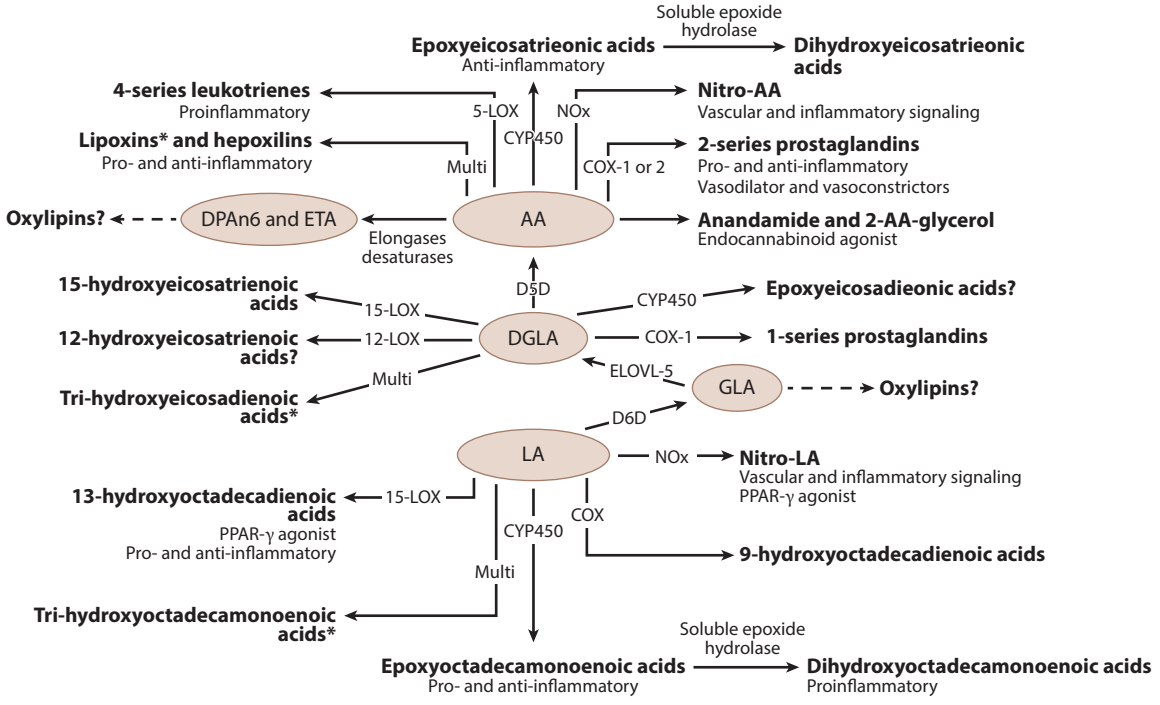


Figure 4

Overview of metabolites derived (or likely to be) from LA. Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; CYP, cytochrome; D5(6)D, δ-5(6) desaturase; DGLA, dihomo-γ-linolenic acid; DPAn6, docosapentaenoic acid (22:5 n-6); DTA, docosatetraenoic acid (22:4 n-6); ELOVL, elongase; ETA, eicosatrienoic acid (20:3 n-6); LA, linoleic acid; LOX, lipoxygenase; Multi, multiple enzymes involved; PPAR, peroxisome proliferation-activated receptors. *As triols, these are analogous to the resolvins and protectins derived from the long-chain omega-3 fatty acids. Adapted from Reference 51.

eicosanoids (8, 118, 119). However, more recent evidence has refuted this oversimplified assertion that ignores the complexity of the entire metabolome of AA. Among a variety of AA metabolites, some are proinflammatory, but others are anti-inflammatory (18, 49, 51). For example, LA can be directly converted to certain cardio-protective derivatives, such as nitrated LA (LNO_2) (18), and some AA metabolites may play a role in the resolution of inflammation (**Figure 4**) (49, 51). There is no clinical evidence that increasing intake of n-6 PUFA leads to increased proinflammatory cytokines in humans (49). Higher intake of n-6 PUFA was not associated with inflammatory biomarkers such as C-reactive protein, interleukin-6, and soluble TNF receptors 1 and 2 in our previous study (116), whereas plasma n-6 PUFA concentration was inversely associated with the level of proinflammatory interleukin-1Ra and positively associated with the level of anti-inflammatory transforming growth factor-β (31).

Most prospective cohort studies have found an inverse association between LA intake and CHD risk. A recent meta-analysis based on 13 cohort studies with a total of 310,602 individuals and 12,479 CHD events found that higher LA intake was associated with a 15% lower risk of CHD events (RR = 0.85; 95% CI, 0.78–0.92) and a 21% lower risk of CHD deaths (RR = 0.79; 95% CI, 0.71–0.89) (**Figure 5**) (30). More importantly, this analysis specified the comparison nutrition for LA: When substituted for either SFA or total carbohydrate, higher LA intake was related to lower risk of CHD. In a meta-analysis conducted by Harris et al., blood/tissue LA

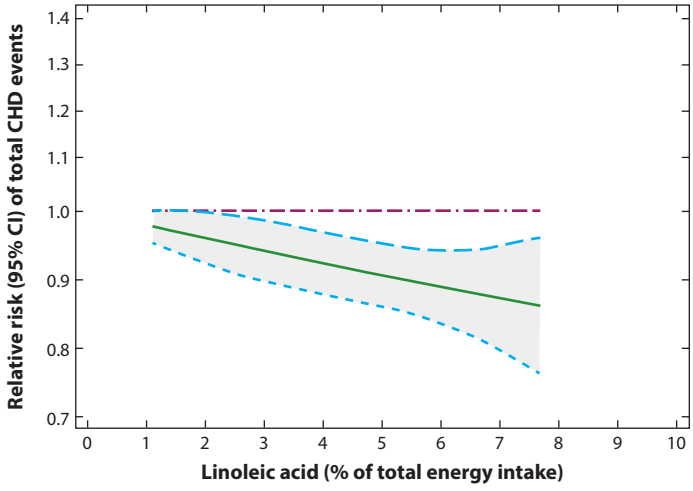


Figure 5

Dose-response analysis for curvilinear association between dietary intake of linoleic acid and total CHD events. $P = 0.91$ for nonlinearity relationship, indicating a linear relationship. Abbreviations: CHD, coronary heart disease; CI, confidence interval. Adapted from Reference 30.

concentrations, biomarkers of LA intake, were inversely associated with nonfatal CHD endpoints (50). Evidence on intakes of n-6 PUFA and stroke is limited. Prospective cohort studies have generally found nonsignificant associations between n-6 PUFA intake and ischemic (38, 52, 63) or hemorrhagic (52, 61, 63) stroke or stroke mortality (128). However, Iso et al. reported a significant inverse association in a Japanese cohort between circulating LA level and risk of stroke, particularly ischemic stroke (62).

Several RCTs have evaluated the effects of high-PUFA and low-SFA diets on CHD events (10, 19, 34, 56, 80, 95–97, 119, 120, 123, 144). Because n-6 PUFA, especially LA, is the predominant PUFA in a habitual diet, these RCTs provided direct evidence of the effects on the risk of CHD of replacing SFA with n-6 PUFAs. However, it is challenging to interpret the findings from these RCTs because most studies were limited by multiple methodological issues, such as a short study duration (123), high attrition rate (34, 120), problems in CHD case adjudication (56), low or insufficiently evaluated participant compliance (10, 56), and the intervention diet confounded by *trans* fatty acid (TFA) (119). Two earlier RCTs in institutionalized populations (19, 95, 96, 135) are among several high-quality RCTs with fewer aforementioned limitations. The Wadsworth Hospital and Veterans Administration Center Study conducted by Dayton et al. is a double-blinded well-controlled trial among 846 male veterans, 26% of whom had history of cardiovascular event (19). The intervention diet used PUFA-rich oils, including corn, soybean, safflower, and cottonseed oils, to replace SFA. As a result, LA accounted for 38% and 10% of total fatty acids in the intervention and control diets, respectively. During an average follow-up of 8 years, the RR for the primary outcome, sudden death or ischemic heart disease, comparing intervention versus control diets was 0.74 (95% CI, 0.53–1.03). In addition, the intervention diet reduced the incidence of stroke by 41% (RR = 0.59; 95% CI, 0.30–1.15) and total CVD by 32% (RR = 0.68; 95% CI, 0.52–0.91). The Finnish Mental Hospital Study was an RCT that included both primary and secondary prevention designs (95, 96, 135). It tested the effect of n-6 PUFA from soybean oil versus SFA, mainly from dairy, in 1,222 patients at psychiatric hospitals with an average follow-up of 6 years. A unique feature of this study was that the compliance of intervention was confirmed by a large increase in LA

concentration in adipose tissue. As a result, the high-PUFA intervention diet, compared with the high-SFA control diet, reduced CHD outcome by 41% (RR = 0.59; 95% CI, 0.47–0.74). Besides these two early RCTs, other appropriately designed RCTs, such as the Oslo Diet-Heart Study (80) and British Medical Research Council Study (97), also consistently observed that increased PUFA consumption, as a replacement for SFA, protected against CHD. In a meta-analysis summarizing evidence from eight RCTs, each 5% increase in energy from PUFA, predominantly n-6 PUFA, in place of SFA led to a 10% reduction in CHD risk (RR = 0.90; 95% CI, 0.83–0.97) (105).

Ramsden et al. recovered and reanalyzed data from the decades-old Sydney Diet Heart Study (SDHS) and the Minnesota Coronary Survey (MCS) and included updated meta-analyses of published data from other RCTs (119, 120). Their findings questioned the cardiovascular benefits of the intake of n-6 PUFA, particularly LA. The SDHS was designed to investigate the effects of increasing LA alone and LA in combination with α-linolenic acid (ALA) on CHD mortality in men with recent coronary events using a single-blinded, randomized controlled design. Ramsden et al. (119, 120) reported that LA intake alone increased the risk of CHD mortality by 74% (RR = 1.74; 95% CI, 1.04–2.91). In addition to limitations such as small sample size and brief duration of the intervention (median followup of 39 months), another major problem with the SDHS is that a high-TFA margarine was included in the high-PUFA diet. Partial hydrogenation of vegetable oils was common in the 1960s, but the deleterious effect of TFA was not yet recognized. The MCS compared high-PUFA versus high-SFA diets in patients hospitalized for mental illness. Reanalysis by Ramsden et al. concluded that "no evidence of benefit in the intervention group for coronary atherosclerosis or myocardial infarcts," but a higher total mortality was observed in the intervention group (120). However, the MCS created an intervention diet by removing almost all natural fats and replacing these with corn oil, resulting in a diet with LA content well above the levels consumed by the US population and any dietary recommendations. The intervention diet in the MCS was likely also confounded by TFA contained in lightly hydrogenated corn oil margarine. More importantly, interpretation of the MCS was complicated by massive dropouts (75% of enrolled participants were lost within the first year) and the very brief duration on the intervention diets (~25% of the study participants received the study diets for more than 1 year). Ramsden et al. (119) also included meta-analyses in their two reports and concluded that replacing SFA with n-6 PUFA had nonsignificant effect on total and CVD mortality, but a beneficial effect on CVD death when SFA was replaced with vegetable oils high in LA with a small amount of n-3 PUFA. However, including recovered data from the SDHS and MCS and excluding several high-quality RCTs were major limitations of the updated meta-analyses. They were also underpowered to reach any conclusions regarding the effect of n-6 PUFA intake on CVD mortality; the wide CI of pooled RR included a potential important benefit. These important limitations called into question the conclusions drawn from the reanalyses of the SDHS and MCS and their associated meta-analyses.

Taken together, consistent evidence from controlled feeding trials of serum lipid as well as prospective cohort studies and high-quality RCTs of clinical endpoints strongly support the association of high intake of n-6 PUFA (predominantly LA), particularly in substitution for SFA, with lower risk of CHD. In our recent analysis of the NHS and HPFS cohorts, higher intakes of LA, compared with either SFA or carbohydrates, were associated with lower total and CVD mortality (142). There is little evidence that higher intakes of LA were associated with increased risk of cancer mortality in humans.

n-3 POLYUNSATURATED FATTY ACID

An n-3 PUFA is a fatty acid with more than one double bond and the first double bond at the third carbon from the methyl terminus. The major dietary n-3 PUFAs include plant-derived ALA and

long-chain n-3 PUFAs, including eicosapentaenoic acid and docosahexaenoic acid from fish and other seafood. The research interest on the cardiovascular effects of n-3 PUFA was stimulated by an early observation of favorable lipid profile and low CHD risk in native people living in the northern part of Greenland who consumed a high amount of fish (5). Three major physiological effects of n-3 PUFA, i.e., anti-inflammation, myocyte electrophysiology, and cell membrane fluidity, have been extensively studied (20, 45, 47). First, n-3 PUFA can be converted to biologically active signaling molecules, such as the eicosanoids (including prostaglandins, thromboxanes, and leukotrienes), epoxides, diols, and ketones, through pathways that involve reactions catalyzed by cyclooxygenases and lipoxygenases, and to nitro-fatty acids through nitration (20). These molecules play important roles in suppressing inflammation and actions promoting the resolution of inflammation. Second, without undergoing metabolism, free n-3 PUFAs can directly influence ion channels on the cell membranes and acutely affect myocyte electrophysiology. Third, incorporation of n-3 PUFAs into cell membranes alters membrane fluidity and interferes with the functions of membrane-associated proteins and hormone-receptor binding, exerting slow-onset and long-lasting effects such as anti-inflammation and antiatherogenesis (20, 107). Through one or more of these mechanisms, n-3 PUFA intake influences multiple CVD risk factors in a dose- and time-dependent manner. N-3 PUFA, when consumed at dietary doses and habitually, showed modest effect of lowering serum triglyceride (46), resting heart rate (100), and blood pressure (37). N-3 PUFA acutely affects ion channels on the cell membranes and therefore possesses potential antiarrhythmic effect, which may be an underlying mechanism of its effect in preventing sudden cardiac death (14, 88).

The effects of long-chain n-3 PUFA intake on CHD outcomes have been studied in both prospective cohort studies and RCTs. Meta-analyses of prospective cohort studies generally suggest a lower risk of CHD among baseline CHD-free participants with higher long-chain n-3 PUFA intake (16, 94) or fish consumption (94); the RR comparing the highest to the lowest quantiles of intake was 0.81 (95% CI, 0.70–0.92) for fish and 0.86 (95% CI, 0.75–0.97) for n-3 PUFA (94). The inverse associations were stronger for CHD death than nonfatal CHD (48, 53). RCTs mostly tested the effect of long-chain n-3 PUFAs as supplements in patients with history of CVD and yielded mixed results. A recent meta-analysis based on 17 RCTs with 4,974 CHD events among 76,580 participants reported a summary RR of 0.94 (95% CI, 0.86–1.03) comparing intervention with control groups (16). By contrast, findings from some meta-analyses indicated a significant risk reduction in the intervention group with n-3 PUFA supplementation for sudden cardiac death or death due to cardiac causes in RCTs (79). The inconsistencies raised the question whether early estimates of the cardio-protective effects of n-3 PUFA were too optimistic.

However, to interpret the RCTs on fish oil supplementation, particularly recent ones, several limitations should be noted. First, most RCTs were among high-CVD-risk population or patients with existing CVD who were already receiving highly efficacious medications such as antihypertensive drugs and statins (127), and it is a challenge to detect a potentially small benefit of fish oil supplements over and above the effects of these drugs. Second, recent RCTs were substantially underpowered because of the lower than expected incidence of events (36, 121). Third, the duration of some RCTs was too short for the changes in n-3 PUFA intake to equilibrate with tissue fatty acid level (121). Finally, given the evidence for a threshold effect of n-3 PUFA intake, an additional low-dose n-3 PUFA supplementation was expected to have minimal effect on CHD death in populations with high background long-chain n-3 PUFA intake (74).

With respect to stroke, a meta-analysis of eight prospective cohort studies comparing the highest with the lowest categories of fish consumption indicated a lower risk of total stroke (RR = 0.69; 95% CI, 0.54–0.88); the inverse association was stronger for ischemic stroke (RR = 0.65; 95% CI, 0.46–0.93) versus hemorrhagic stroke (RR = 0.80; 95% CI, 0.44–1.47) (54). However, long-chain n-3 PUFA from either dietary intake or supplementation was not appreciably associated

with stroke risk (15, 55). Fish consumption or long-chain n-3 PUFA intake was inversely associated with the incidence of heart failure in several prospective cohort studies (7, 28, 82, 83, 99). The effect of long-chain n-3 PUFA on heart failure has not been directly tested in RCTs, but some supporting, albeit indirect, evidence was found in the GISSI-HF trial (testing effects of n-3 PUFA and rosuvastatin on mortality-morbidity of patients with symptomatic cardiac heart failure) (134). This placebo-controlled RCT found long-chain n-3 PUFA supplementation significantly reduced total mortality by 9% (RR = 0.91; 95% CI, 0.83–1.00) and improved the left ventricular ejection fraction. Although some evidence from mechanistic studies indicated higher long-chain n-3 PUFA intake may reduce risk of atrial fibrillation (AF) (76, 124), results from prospective studies analyzing incidence of AF were mostly null (9, 35, 40, 75, 106, 122). Several RCTs evaluated the effect of long-chain n-3 PUFA on AF recurrence among patients with reverted or postoperative AF. A meta-analysis of these trials showed no significant effects of long-chain n-3 PUFA on AF recurrence (RR, 0.95; 95% CI, 0.79–1.13) or on postoperative AF (0.86; 95% CI, 0.71–1.04) (87).

Compared with long-chain n-3 PUFA, the effects of the plant-based n-3 PUFA, ALA, on CVD risk were less characterized. Current evidence indicates ALA has similar favorable effects on lipid profile to those of LA (85). A recent meta-analysis indicated dietary ALA intake was associated with a modestly lower risk of total CVD; the pooled RR comparing the highest with the lowest intake levels was 0.90 (95% CI, 0.81–0.99) (113). The Alpha Omega Trial compared the effect of supplemental ALA (2 g/day) with oleic acid on CVD and reported nonsignificant results (RR = 0.91; 95% CI, 0.78–1.05) (74). However, the number of events in this trial was modest, and the wide CI of RR could include an important beneficial effect. The Lyon Diet Heart Study that tested the effect of a Mediterranean-style diet with high ALA content versus a low-fat control diet found a 70% reduction in recurrence of CHD in the intervention group compared with the control group (21). However, the observed beneficial effect may not be entirely attributed to high ALA intake because the intervention also included advice for modification of other dietary components.

Epidemiological studies that employed circulating n-3 PUFAs as objective biomarkers of intake level generally yielded inverse associations with CHD risk. A recent pooling project including 19 prospective cohorts found that higher plasma levels of docosahexaenoic acid, docosapentaenoic acid, and ALA were associated with significantly lower risk of CHD death (25). In a meta-analysis, the pooled RRs for CHD (95% CI) comparing the top versus bottom third of fatty acid distribution are 0.93 (0.83–1.03) for ALA, 0.87 (0.78 to 0.97) for total long-chain n-3 PUFAs, 0.64 (0.47–0.89) for docosapentaenoic acid, 0.79 (0.67–0.93) for docosahexaenoic acid, and 0.78 (0.65–0.94) for eicosapentaenoic acid (16). Similar to the observations on dietary n-3 PUFA intake and n-3 PUFA supplementation, the associations of biomarkers were stronger for CHD death (103). The results of circulating n-3 PUFA and stroke were limited and inconsistent (15, 103); a meta-analysis based on four prospective studies comparing the highest with the lowest n-3 PUFA levels yielded an RR of 1.04 (95% CI, 0.90–1.20) (15). Several lines of evidence support the inverse association of circulating total long-chain n-3 PUFA with heart failure (104, 159) and AF (131, 140, 155), whereas evidence regarding association between circulating ALA and heart failure is conflicting (78, 145).

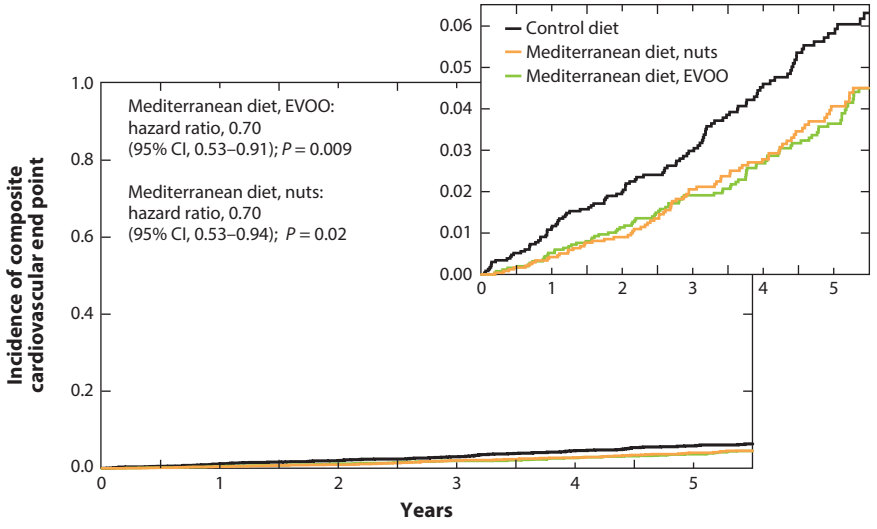
Taken together, there is concordance of evidence from mechanistic studies, controlled feeding trials, and prospective studies of dietary intakes and biomarkers that n-3 PUFA, including ALA and long-chain n-3 PUFAs, is protective against CHD, especially CHD death. However, the beneficial effects of fish oil supplementation are not consistent in reducing CVD mortality. Nevertheless, several limitations of the RCTs are worth noting, for example, background dietary n-3 PUFA, widely used antihypertensive medications and statins, and limited statistical power of several RCTs. Therefore, current recommendations for primary prevention of CVD have focused on regular consumption of fatty fish rather than on taking fish oil supplements.

MONOUNSATURATED FATTY ACIDS

MUFAs are fatty acids with only one double bond. The most common MUFA in the human diet is oleic acid (~90% of all MUFAs). Early interest in the effect of MUFA on CVD stems from the observation in the Seven Countries Study that certain Mediterranean populations had a low prevalence of CHD (70). A traditional Mediterranean diet is high in MUFA (16% to 29% of total energy intake) mainly owing to high consumption of olive oil. Controlled feeding studies found that substituting MUFA for carbohydrate lowers total and LDL cholesterol and increases HDL cholesterol, therefore decreasing the TC to HDL ratio and predicting a lower risk of CVD (93). MUFA intake also favorably affects other CVD risk factors, including factors related to thrombogenesis, in vitro LDL oxidative susceptibility, insulin sensitivity, and inflammatory makers (72, 90, 111, 154).

Although MUFA intake, when substituted for carbohydrate, showed favorable effect on lipid profile, MUFA intake was generally not associated with risk of CHD in prospective cohort studies that specified total carbohydrate as a comparison nutrient (59, 64, 84) or those without explicit specification of a comparison nutrient (16, 129). An explanation for this discrepancy is the strong correlations between MUFA and SFA due to shared food sources such as dairy and meats in most Western diets. In our recent study, MUFA intake, when compared with total carbohydrate, was associated with significantly lower risk of CVD and total mortality during an up to 32-year follow-up; HR for replacing 5% of energy from carbohydrate by the equivalent energy from MUFA was 0.90 (95% CI, 0.83–0.98) for CVD mortality and 0.90 (95% CI, 0.87–0.94) for total mortality (142). These findings were different from the null or even positive associations observed in previous studies, because in our cohorts the major food sources of MUFA were shifted over time from animal-sourced foods, mainly red meat, to plant-sourced foods, such as olive oil and nuts (142). Similar to our findings, Guasch-Ferré et al. reported that intake of MUFA, when compared with carbohydrate, was inversely associated with total CVD risk (HR = 0.63; 95% CI, 0.43–0.94) in the PREDIMED trial, possibly because olive oil was the main food source of MUFA in this population (41). Prospective cohort studies also showed that the intake of MUFA, when substituted for SFA, was related to lower CVD risk. Our recent work in the NHS and HPFS found that replacing 5% of energy from SFAs with 5% of energy from MUFAs was associated with a 15% lower risk of CHD (HR = 0.85; 95% CI, 0.74–0.97) (84). In a meta-analysis, the ratio of MUFA to SFA was associated with a significantly lower risk of CVD death (RR = 0.91; 95% CI, 0.83–0.99) and a lower risk of total CVD that approached significance (RR = 0.93; 95% CI, 0.86–1.01) (129). The evidence was inconclusive for stroke; a recent meta-analysis found no association of MUFA intake, without specification of comparison nutrient, with total and ischemic stroke, but a suggestive inverse association with hemorrhagic stroke (13).

Olive oil, as an abundant food source of MUFA, has also been linked to lower CVD risk in several studies. A recent meta-analysis based on prospective cohort studies reported that an increase in olive oil intake of 25 g/day was associated with an 18% reduction in total CVD risk (RR = 0.82; 95% CI, 0.70–0.96) (89). Interestingly, the inverse association appeared to be stronger for stroke than CHD (89, 129). The landmark PREDIMED trial tested two Mediterranean dietary patterns, supplemented with either extra virgin olive oil or mixed nuts, versus a control diet, for primary prevention of CVD among 7,447 men and women free of diagnosed CVD but with high CVD risk at baseline (29). During a median of 4.8 years of follow-up, the intervention diets significantly reduced major CVD events (myocardial infarction, stroke, and CVD deaths) by approximately 30%, compared with the control diet (HR = 0.70; 95% CI, 0.54–0.92 for the Mediterranean diet supplemented with extra virgin oil group and HR = 0.72; 95% CI, 0.54–0.96 for the Mediterranean diet supplemented with nuts) (**Figure 6**). This trial has provided the



Number at risk

Control diet	2,450	2,268	2,020	1,583	1,268	946
Mediterranean diet, EVOO	2,543	2,486	2,320	1,987	1,687	1,310
Mediterranean diet, nuts	2,454	2,343	2,093	1,657	1,389	1,031

Figure 6

Kaplan-Meier estimates of the incidence of primary endpoint (a composite of acute myocardial infarction, stroke, and death from cardiovascular causes) in the PREDIMED Trial. Abbreviations: CI, confidence interval; EVOO, extra virgin olive oil. Adapted from Reference 14.

strongest evidence to date supporting the health benefits of a Mediterranean diet rich in olive oil or nuts in primary prevention of CVD.

TRANS FATTY ACIDS

Industrially produced TFAs (unsaturated fatty acids with one or more carbon double bonds in the *trans* configuration) are formed when vegetable oils are partial hydrogenated to form margarine and shortening. Consistent evidence from controlled feeding studies shows that TFAs, when substituted for SFA or unsaturated fatty acids, raise LDL cholesterol, reduce HDL cholesterol, and greatly raise the TC to HDL ratio, a strong predictor of CVD risk (93). TFAs also adversely affect endothelia function, promote inflammation, reduce the particle size of LDL cholesterol, and reduce the level of lipoprotein(a), each of which further increases the risk of CVD (102).

The elevated risk of CHD in relation to a higher intake of TFA was first reported in the NHS (151) and confirmed by following prospective cohort studies (3, 115, 156). In a meta-analysis, the summary RR comparing the top and bottom thirds of TFA intake was 1.16 (95% CI, 1.06–1.27). Studies using biomarkers of TFA also found that both circulating and adipose tissue TFAs were positively associated with CHD risk (1, 6, 17, 132). Different TFA subtypes (of varying chain length and unsaturation) and isomers (of similar chain length and unsaturation, but different location of double bonds) have been studied. Current data using red blood cells or adipose tissue show that the 18-carbon *trans* isomers are relevant to increased CHD risk: Compared with 18:1 *trans* isomers, 18:2 *trans* isomers are more strongly associated with CHD risk (6, 77, 132, 143). A recent study conducted by Wang et al. (143) further investigated different 18:2 *trans* isomers in

more detail. They observed that *trans*/*cis* and *trans*/*trans* 18:2 *trans* isomers are each significantly associated with higher risk of CHD, whereas *cis*/*trans* 18:2 is not associated with CHD risk.

As industrially produced TFAs are being phased out of the US food supply and plasma TFA levels have decreased substantially over time (138), interest in TFAs from ruminant fats has increased. To date, most prospective studies have not documented significant associations between either intake (65, 110, 115, 151) or biomarkers (23, 133, 157) of these TFAs from ruminant fats and CVD risk, but a recent cohort study in the Ludwigshafen Risk and Cardiovascular Health Study reported an inverse association between level of *trans*-16:1n-7 in erythrocyte membranes and risk of CVD mortality and sudden cardiac death (71). However, interpreting these findings is complicated owing both to omission of important confounders, e.g., dietary variables and blood pressure, in statistical modeling and to a potential reverse-causation issue because the cohort consisted of participants hospitalized for coronary angiography. Some investigators postulated that TFAs from dairy, particularly *trans*-16:1n-7, may have unique biological functions and favorable cardiometabolic effects such as reduction of hepatic fat synthesis and increase of muscle insulin sensitivity (158). However, given the high correlation between *trans*-16:1n-7 and dairy intake, it is challenging to distinguish the effects of these TFAs from the effects of other components of dairy foods, such as vitamin D, minerals, gangliosides, and bioactive peptides.

METHODOLOGICAL CONSIDERATIONS IN STUDIES ON DIETARY FATTY ACIDS AND CARDIOVASCULAR DISEASE

To measure habitual dietary intakes, the most common choice in large prospective cohort studies is a food frequency questionnaire (FFQ) that assesses the usual frequency of participants' intake of each food item over a previous certain time period. The advantage of FFQs over other methods such as 24-hour recalls or diet records is that they assess long-term usual dietary intake and have relatively lower random within-person variation. Also, FFQs can be administered in very large cohorts because of their low cost and low participant burden. Measurement errors are inevitable in any self-reported dietary assessment tool. However, the use of repeated measurements of diet can help dampen within-person variation and reflect long-term dietary habits. The FFQs used in the NHS and HPFS have been extensively validated against multiple-day diet records and biomarkers. For example, correlations between energy-adjusted intakes assessed by the 1986 FFQ and the mean of multiple weighed 1-week dietary records collected in 1980 and 1986, corrected for variation in the records, were 0.67 for total fat, 0.70 for SFA, 0.69 for MUFA, and 0.64 for PUFA (147, 148). Correlation between dietary fatty acid intake assessed by the FFQ and the composition of fatty acids in adipose tissue was 0.51 for TFA, 0.35 for LA, and 0.48 for long-chain n-3 PUFA in the NHS (86), and 0.29 for TFA, 0.48 for LA, and 0.47 for eicosapentaenoic acid in the HPFS.

Assessing objective biomarkers of dietary fatty acids in epidemiological studies is particularly useful in minimizing measurement errors resulting from self-reported diets. However, sensitive or specific biomarkers exist for only several fatty acids that cannot be endogenously produced. In addition, biomarkers of fatty acid intake in plasma or adipose tissue reflect percentages of total fatty acids, rather than absolute amounts (147, 148). Moreover, because of their high cost, the use of fatty acid biomarkers has been limited to relatively small studies. Therefore, assessments of biomarkers of fatty acids are useful complements, rather than replacements, to the data from validated FFQs.

Among various study designs, RCTs are often considered as the gold standard for investigating the effect of diet on disease risk because they can eliminate issues such as confounding and

selection biases. However, because of the methodological limitations discussed above, e.g., poor compliance and high drop-out rates, long-term RCTs testing the effects of dietary interventions on hard endpoints such as CVD incidence are extremely difficult to conduct. High cost and ethical considerations are additional challenges for conducting such a trial. Thus, in most situations, large prospective cohort studies of hard clinical endpoints, when well designed, can provide the best available evidence to inform dietary recommendations.

Meta-analyses, when conducted and interpreted carefully, can be useful for summarizing a large amount of evidence and advancing the field. However, many authors have rushed the publication of their meta-analyses without content knowledge and insufficient understanding of underlying biological mechanisms, leading to incomplete literature searches, flawed analytic methods, and misleading conclusions. A more powerful study design for summarizing evidence is a pooled analysis of individual-level data from multiple studies, allowing for standardized definition of dietary exposures and controlling for confounding factors. For example, Jakobsen et al. conducted a pooled analysis of 344,696 participants from 11 prospective cohort studies in the United States, Europe, and Israel with 5,249 CHD events and 2,155 CHD deaths, which estimated the effects of replacing SFA with PUFA and showed clear benefits for reducing CHD risk (64).

Although this review primarily focuses on dietary fatty acids, CVD has multiple interacting dietary determinants, including a matrix of foods and overall dietary pattern, rather than several single nutrients. Also, the emphasis of current dietary guidelines has moved from the adequacy of nutrients to the adoption of healthy dietary patterns. It is therefore essential to incorporate the recommendations on dietary fat intake into guidelines based on foods and dietary patterns; practically, this will be achieved by recommending a dietary pattern with high consumption of foods rich in unsaturated fatty acid, such as vegetables, plant oil, nuts, and fatty fish, but low consumption of SFA-rich foods, such as red meat and processed foods. However, nutrient-based recommendations still have a role in modern dietary guidelines, as has been exemplified by the recommendation of the 2015–2020 Dietary Guidelines for Americans to retain the 10% upper limit on dietary SFA. This limit is justified because SFAs can be easily added to foods, meals, and habitual diets in the form of cooking fats as well as processed foods to enhance a food's shelf life while increasing palatability. Therefore, a focus on limiting SFA intake covers a wide range of foods that individuals need to be aware of in making healthy food choices. Also, limiting SFA intake can help set standards for food industry and government programs for nutrition assistance, such as the National School Lunch Program and the Supplemental Nutrition Assistance Program. Further, certain nutrients, e.g., TFA, may affect CVD risk independent of an overall dietary pattern. For TFA, a focus on one single nutrient has been important for the development of a regulatory ban and legislation.

SUMMARY AND PRACTICAL PERSPECTIVES

In summary, there is compelling evidence that different types of dietary fatty acid have divergent effects on CVD risk and that the type of fat is more important than total amount of fat. The effect of a specific fatty acid depends strongly on the source of calories with which it is compared. Little or no cardiovascular benefits were seen when SFA is replaced by total carbohydrate, but a significant reduction in CVD risk is achieved when SFA is replaced by MUFA and/or PUFA. The effects of different food sources of SFA and different lengths of SFA warrant further study. Although higher intake of TFA from hydrogenated vegetable oils has adverse effects on lipid profile and CVD risk, the effects of *trans* isomers from ruminant fat remain controversial. Abundant evidence from RCTs and prospective cohort studies support the benefits of both n-6 and n-3 PUFAs, including

both plant-sourced ALA and long-chain n-3 PUFAs from fish, in reducing CVD risk, but the benefits of fish oil supplements have not been clearly demonstrated. The ratio of n-6:n-3 PUFA was a less relevant indicator than absolute amounts of these fatty acids in the context of dietary prevention of CVD, as epidemiologic studies have found little relationship between dietary n-6:n-3 ratio and CVD endpoints or between plasma n-6:n-3 ratio and CVD risk factors (31, 69, 112, 142). The totality of current evidence supports contemporary Dietary Guidelines for Americans recommendations of consuming less than 10% of calories from SFA and replacing SFA with unsaturated fatty acids (136). Following these recommendations can have substantial impact on global population health. In a recent report from the Global Burden of Disease project, modifying the nonoptimal intakes of n-6 PUFA, SFA, and TFA would prevent an estimated 10.3%, 3.6%, and 7.7% of CHD deaths worldwide (32).

Findings from nutritional epidemiologic studies, together with evidence from intervention studies on intermediate biomarkers, have played a critical role in informing health policy (136). A successful example is the elimination of TFA from the US food supply. Since 2006, the US Food and Drug Administration has required the TFA content of food to be included in nutrition facts labels in response to robust evidence of the adverse effect of TFA on lipid profile (91–93) and CVD risk (59, 102, 151). Also, many states and cities have taken legislative/regulatory action to limit TFA use in restaurants and other food service locations (108). Most manufacturers have reformulated products to reduce the amount of TFA (27, 101). Most recently, the Food and Drug Administration released its final determination that partially hydrogenated oils were not generally recognized as safe for use in food. These policies have led to a large reduction in TFA in the US diet, contributing to approximately half of the improvement in overall dietary quality and a substantial reduction in disease burden from 1999 to 2012 in the US population (141).

Based on current evidence, several practical recommendations have been made by the 2015 Dietary Guidelines Advisory Committee to improve quality of dietary fats. First, nonhydrogenated vegetable oils that are high in unsaturated fats and relatively low in SFA (e.g., soybean, corn, olive, and canola oils) instead of animal fats (e.g., butter, cream, beef tallow, and lard) or tropical oils (e.g., palm, palm kernel, and coconut oils) should be the primary source of dietary fat. Second, dietary advice should put emphasis on optimizing types of dietary fat and not reducing total fat. Consumption of "low-fat" or "nonfat" products with high amounts of refined grains and added sugars should be discouraged. Finally, when their consumption is reduced, refined carbohydrates and added sugar should not be replaced with foods high in saturated fat. Instead, refined carbohydrates and added sugar should be replaced by healthy sources of carbohydrates (e.g., whole grains, legumes, vegetables, and fruits) and healthy sources of fats (e.g., nonhydrogenated vegetable oils that are high in unsaturated fats as well as nuts and seeds). These recommendations are consistent with those made by the Presidential Advisory from the American Heart Association (123a). Following these recommendations has the great potential to improve overall dietary quality and reduce risk of CVD and other chronic diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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