

DIETARY n-6 AND n-3 FATTY ACID BALANCE AND CARDIOVASCULAR HEALTH

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Key Words linoleic acid, linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, coronary heart disease, cardiovascular risk factors

■ **Abstract** Epidemiological and clinical studies have established that the n-6 fatty acid, linoleic acid (LA), and the n-3 fatty acids, linolenic acid (LNA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) collectively protect against coronary heart disease (CHD). LA is the major dietary fatty acid regulating low-density lipoprotein (LDL)-C metabolism by downregulating LDL-C production and enhancing its clearance. Further, the available mass of LA is a critical factor determining the hyperlipemic effects of other dietary fat components, such as saturated and trans fatty acids, as well as cholesterol. By contrast, n-3 fatty acids, especially EPA and DHA, are potent antiarrhythmic agents. EPA and DHA also improve vascular endothelial function and help lower blood pressure, platelet sensitivity, and the serum triglyceride level. The distinct functions of these two families make the balance between dietary n-6 and n-3 fatty acids an important consideration influencing cardiovascular health. Based on published literature describing practical dietary intakes, we suggest that consumption of ~6% en LA, 0.75% en LNA, and 0.25% en EPA + DHA represents adequate and achievable intakes for most healthy adults. This corresponds to an n-6/n-3 ratio of ~6:1. However, the absolute mass of essential fatty acids consumed, rather than their n-6/n-3 ratio, should be the first consideration when contemplating lifelong dietary habits affecting cardiovascular benefit from their intake.

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INTRODUCTION

Polyunsaturated fatty acids (PUFAs) of the n-6 and n-3 series are essential nutrients that exert an important influence on plasma lipids and serve cardiac and endothelial functions to impact the prevention and treatment of coronary heart diseases (CHD). Both n-6 and n-3 PUFAs have distinct biological effects contributing to their cardioprotective action. While it is accepted that PUFAs of both series are dietary essentials, both the absolute intakes (g/d) and the n-6/n-3 ratio required to achieve optimal CHD health benefits are somewhat controversial, in part due to the failure to consider their intake in the context of total daily fat and total daily PUFA (i.e., as a percent of energy) consumed by the population under study. This review critically evaluates the role of n-6 and n-3 PUFA on cardiovascular risk with consideration given to the "ideal" balance between the two series of fatty acids. A case is made that the absolute mass of the n-6 and n-3 PUFA consumed, rather than the ratio of n-6/n-3 PUFA, should be the main consideration in the prevention and management of CHD. We emphasize that our focus is on the day-to-day consumption of a healthy amount of essential fatty acids, not on their potential as corrective therapy for individuals with CHD.

POLYUNSATURATED FATTY ACIDS AND CORONARY HEART DISEASE RISK

Linoleic Acid

Linoleic acid (LA, 18:2n-6) is the primary (in terms of mass consumed) essential fatty acid and represents the basis of the n-6 family. Its predominance reflects the fact that it is the most common PUFA incorporated into the dynamic phospholipids (especially lecithin) needed for membrane structure and lipoproteins [particularly phospholipid-rich high-density lipoprotein (HDL)] involved in lipid transport. It can be elongated and desaturated to form 20 carbon fatty acids, such as arachidonic acid (AA, 20:4n-6), which together function via structural lipids and cell signals to control numerous cell activities responsible for daily living. In fact, epidemiological evidence supports a role for dietary LA in reducing the risk of CHD. A cross-population study (88) in healthy men from four European populations found that higher adipose tissue LA, a marker of dietary LA intake, was associated with lower CHD mortality. Further, serum linoleic acid was negatively related to cardiovascular death in postinfarction middle-aged men (104), while low dietary LA intake predisposed to myocardial infarction (98). Hegsted & Ausman (45) studied dietary factors impacting CHD in men from 18 countries (with PUFA intake ranging from 1.4% to 11% en) and found that dietary PUFA was negatively associated with CHD mortality after adjusting for saturated fatty acid (SFA) intake. In the multicenter population from the National Heart, Lung, and Blood Institute Family Heart Study (27), the highest tertile of LA intake (11.7 g/d, or about 5% en at

2000 kcal/d energy intake) was associated with a 39% lower prevalence odds ratio for coronary artery diseases (CAD) compared to the lowest tertile of LA intake (3.9 g/d, or about 1.7% en), when adjusted for LNA intake and other relevant risk factors. A recent prospective nested case-control study of Japanese subjects (52) found reduced serum LA levels in patients with ischemic stroke compared to healthy controls, suggesting that higher dietary PUFA intake may protect against this type of stroke. Additionally, higher dietary LA intake was associated with lower risk of type 2 diabetes mellitus in women (90).

EFFECT OF LINOLEIC ACID ON LIPOPROTEINS LA is the most potent dietary fatty acid for reducing plasma total cholesterol (TC) and LDL-C (43, 46, 71), each of which is an established CHD risk factor. Initial human clinical studies (4, 9, 11, 46, 67) clearly demonstrated that diets extremely high in LA, i.e., providing 16% to 29% en as LA, significantly lowered plasma LDL-C by 16%–22% compared to diets high in saturated fatty acids (19% to 30% en SFA) and low in LA (only 2.3%–4.5% en LA). This double shift in fatty acids, resulting in simultaneous changes in LA and SFA, is a recurrent confounding theme in many clinical and epidemiological studies on the subject. As a consequence, interpretation is compromised because one cannot discern whether the increased LA or the removal of SFA provided the greater contribution toward lowering TC (44). Similarly, confusion exists over the relative potency of LA-rich and oleic acid (OA)-rich fats for lowering LDL and improving the LDL/HDL ratio, because multiple changes in LA, monounsaturated fatty acid (MUFA), and SFA between diets complicate interpretation. For example, the LDL-C lowering potency of LA has been reported to be greater than (11, 25, 48) or similar to (29, 67, 70) that of OA-rich fats. In a direct comparison, Howard et al. (48) reported that replacing OA with 3%–14% en from LA led to a progressive decline in LDL-C and TC in moderately hypercholesterolemic subjects, demonstrating the superiority of LA over OA in this capacity. In a recent meta-analysis of 60 controlled human trials, Mensink et al. (71) found that inserting PUFA (as opposed to MUFA or SFA) for carbohydrates resulted in a significantly greater decline in LDL-C and the ratio of TC to HDL-C, the latter being a sensitive predictor of CHD risk (71). One needs to recall that such meta-analyses will only predict relative fatty acid potency based on the study designs included in the analysis, so that the bias of excluding/including specific studies should be scrutinized carefully when assessing the outcomes.

As for possible mechanisms of LA action, animal studies consistently reveal that LA-rich fats enhance hepatic receptor-dependent clearance of LDL (101, 106) and concomitantly reduce LDL-C production (106). Woollett et al. (106) fed hamsters varying quantities of hydrogenated coconut oil and safflower oil from 0%–20% by weight at a constant cholesterol intake of 0.12%. When LA-rich safflower oil replaced SFA-rich coconut oil, LDL receptor activity increased from 25%–80% of control and the LDL-C production rate decreased by 155%–200%, resulting in a 75% net decline in LDL-C. When Cebus monkeys were fed either 18% en as LA, MUFA, or SFA in a diet containing 30% en as fat and 0.1% cholesterol

in a crossover design, the LDL apolipoprotein B (apoB) fractional clearance rate increased 17% and the LDL production rate declined by 23% during the LA period compared to the SFA period, whereas LDL-C production declined by 26% as the only notable response in MUFA-fed monkeys (16). Again, these hamster and Cebus data are complicated by multiple fatty acid shifts between diet periods, but the inference is that LA is the major dietary fatty acid that clearly enhances LDL-C clearance and reduces its production.

LINOLEIC ACID AS A DETERMINANT OF CHOLESTEROLEMIC EFFECTS OF OTHER FATTY ACIDS To better conceptualize the pivotal role of LA in regulating cholesterol metabolism in concert with other dietary fatty acids, the “18–2 threshold” hypothesis was developed and tested. This hypothesis states that the absolute mass of available LA, both from diet and adipose stores, dictates the cholesterolemic effects of other dietary fatty acids, particularly individual dietary SFA (43, 44). In monkeys and gerbils fed <3% en LA (44, 85, 86), 12:0, 14:0, and to a lesser extent, 16:0 and even 18:1, increased TC and LDL-C in the presence of dietary cholesterol (0.02%–0.08%). However, specific SFA exerted a minimal effect when LA intake exceeded 5% en in these animals, especially at low cholesterol intakes. Furthermore, the main effect of SFA in the absence of dietary cholesterol appears to be on very-low-density lipoprotein (VLDL) and LDL production, rather than clearance (38, 56). Consuming a 16:0-rich diet increased VLDL production and led to increased plasma LDL-C relative to an 18:1-rich diet, at least when LDL receptor activity was depressed by dietary cholesterol and low LA intake prevailed (44, 57). Adding LA to the diet enhanced LDL receptor activity and decreased the LDL production induced by SFA, thus minimizing the hyperlipemic effects of a constant amount of SFA. In essence, if LA intake falls below the “threshold” requirement, which is dictated by a combination of dietary and genetic factors, the hyperlipemic effects of other dietary stressors, including SFA, trans fatty acids, and cholesterol, will be greatly exaggerated. The point is that LA intake (mass) represents a critical consideration in the dietary fat approach to regulating plasma cholesterol.

Alpha-Linolenic Acid

Alpha-linolenic acid (LNA, 18:3n-3) is the second primary essential fatty acid that can be elongated and desaturated to form highly specialized 22-carbon fatty acids within the n-3 family. Recent human studies suggest that dietary LNA (and its subsequent metabolic end products) may protect against CHD (10, 26, 27, 50). A population-based case-control study in Costa Rica found that high adipose tissue LNA content was associated with a lower risk of myocardial infarction (MI) (10). Dietary LNA intake was significantly inversely associated with mortality from CHD and all-cause mortality in the Multiple Risk Factor Intervention Trial (28). Large prospective studies in women (50) and men (7) found that LNA protected against cardiac deaths and nonfatal myocardial infarction, independent of other

relevant factors. The highest quintile of LNA intake (1.36 g/d or 0.6% en LNA mean intake) was associated with 45% fewer cardiac deaths compared to the lowest quintile of LNA intake (0.71 g/d or 0.3% en) in women (50). In the National Heart, Lung, and Blood Institute Family Heart Study involving 4406 men and women (27), the highest quintile of LNA intake (1.1 g/d or 0.5% en LNA mean intake) was associated with 40% lower mortality from CAD compared to the lowest quintile of LNA intake (0.5 g/d or 0.2% en LNA), after adjusting for LA intake and other relevant factors. In addition, dietary intake of LNA was inversely related to carotid atherosclerosis in this study population (26). In the Lyon study (24), threefold higher LNA intake in the experimental group (1.5 g/d or 0.7% en) compared to controls resulted in 70% fewer cardiac deaths following a first myocardial infarction. In a double blind, placebo-controlled study in India (99), 3.6-fold higher LNA intake in the experimental group (2.9 g/d or 1.3% en) compared to controls was associated with significantly lower cardiac deaths and nonfatal myocardial infarction. However, in the latter two studies several dietary factors known to impact CHD risk were varied simultaneously, so causality is less than clear. Two epidemiological studies (81, 96) found no association between LNA intake and CHD risk.

ALPHA-LINOLENIC ACID'S POSSIBLE MECHANISMS The manner in which dietary LNA may reduce CHD risk is not clear, but may have more to do with cardiac function than with plasma lipids. Some studies have inferred that LNA was similar to LA and OA in lowering plasma TC (19, 55, 65, 82). However, in two of these (65, 82), when LNA replaced dietary LA by substituting flaxseed oil for safflower oil, plasma LDL-C decreased only 2% or 8% (at 3.5% en or 5.3% en LNA intake, respectively) compared to 9% and 16% decreases with LA-rich diets (at 6.7% en or 7.8% en LA intake, respectively). This suggests that LNA is not as effective as LA in modulating LDL receptor activity or LDL-C production and clearance.

Protective effects of LNA rather may reflect the impact of n-3 fatty acids on cardiac arrhythmia, inflammation, and thrombosis. In dogs, LNA ethyl ether infusion prevented ischemia-induced ventricular fibrillation, a primary event in sudden cardiac death (15). Rats fed a canola oil-enriched diet (2.6% en LNA and 18% en OA) showed significantly reduced incidence of arrhythmias and cardiac mortality compared to those fed relatively less LNA (0.4% en LNA and 20% en OA) (68). Nestel et al. (78) found enhanced arterial compliance after four weeks when flaxseed oil supplementation provided 9% en LNA (and 2.7% en LA) in exchange for OA in obese subjects, despite increased *in vitro* LDL oxidation. A recent study in dyslipidemic male patients (87) observed that a high-LNA diet (4% en LNA from flaxseed oil) compared to high-LA diet (9% en LA from safflower oil) significantly lowered C-reactive protein, IL-6, and serum amyloid A, all inflammatory markers implicated in atherogenesis. Here, again, the switch between fats was extreme, making interpretation difficult. By contrast, a randomized, placebo-controlled, double blind study found that a modest dietary enrichment with LNA (at 3.5 g/d or 1.6% en as a flaxseed oil supplement) did not alter immune markers or monocyte functional activity in healthy male subjects (105).

Thrombotic events play a significant role in atherogenesis and CHD, but LNA effects on platelet aggregation and thrombosis are inconsistent (58), presumably because the distinction between physiological and pharmacological effects of LNA is blurred. Healthy men consuming a diet supplemented with flaxseed oil (LNA at 8% en) had significantly decreased collagen-induced platelet aggregation compared to those fed a sunflower oil-supplemented diet (12% en LA) (3), which may reflect hyperthrombotic tendencies of sunflower oil or a protective influence of flaxseed oil. Again, a realistic mass intake of a specific fatty acid is to be emphasized because extreme dietary fatty acid (fat) comparisons do not represent practical real-life experience or normal physiology. Applying a more practical approach, Freese et al. (33) reported a decrease in platelet aggregation in hyperlipidemic subjects consuming ~2% en as LNA (and 6.5% en LA) compared to a diet providing 0.3% en as LNA (and 8% en LA). However, in a blinded clinical study, no beneficial effects of LNA on platelet adhesion were detected in men with atherosclerosis who were fed 15 g/d (~6% en) LNA (13). Likewise, a randomized blinded clinical trial in men (76) reported that supplementing 5.5 g/d (2.5% en) LNA plus 1.5 g/d LA (0.6% en) did not significantly affect thrombotic events compared to a 6.3 g/d (3% en) LA diet. In moderately hyperlipidemic patients, feeding 4.5 g/d (2% en) or 9.5 g/d (4% en) LNA (at 4% en and 1.5% en LA intake) failed to alter any blood coagulation or fibrinolytic factors compared to a control diet providing 13 g/d (5.7% en) LA and 0.06 g/d (0.03% en) LNA (32). It is not known whether any of the observed effects of LNA on CHD risk factors reflect conversion to its longer-chain metabolic products, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or are independent of the longer-chain polyunsaturated fatty acid (LCP) effects.

Eicosapentaenoic Acid and Docosahexaenoic Acid

Mounting evidence suggests that diets containing modest amounts of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), the conversion products of LNA found primarily in oily fish, decrease the risk of ischemic heart disease. Several population-based studies reported lower incidence of CHD with high fish consumption (8, 47, 61, 80), suggesting that n-3 LCP in fish may be protective against CHD. High serum and adipose tissue n-3 LCP have been associated with reduced risk of acute or fatal MI (63, 72, 83, 96), primary cardiac arrest (100), and sudden cardiac death (1), as well as CAD deaths and all-cause mortality (31). In the Physicians Health Study involving 20,551 males in the United States (2), consumption of one or more servings of fish per week was associated with a 52% lower risk of sudden cardiac death compared to less than one fish meal/month. In the Nurses Health Study (49), consumption of five or more servings of fish per week was associated with 45% fewer cardiac deaths compared to consumption of less than one fish meal/month in 84,688 women. Randomized clinical trials have also shown a protective effect of EPA and DHA on CHD mortality. In the Diet and Reinfarction Trial (18), two-year CAD mortality and total mortality were 30%

lower among post-MI male patients who were advised to consume 200–400 g fatty fish/week than among similar patients who did not receive the advice. The weekly intake of 200–400 g fish in the experimental group corresponded to a combined intake of EPA and DHA of slightly less than 1 g/d. The Italian GISSI-prevention trial (37), involving 11,324 post-MI patients of both sexes, is the only clinical trial that investigated the effects of purified EPA + DHA fatty acids on cardiac endpoints. In that study daily intake of 900 mg EPA + DHA ethyl esters in capsules was associated with 20% lower CAD mortality and 14% lower total mortality at the 3.5-year follow-up. A meta-analysis of 11 randomized controlled trials comparing dietary and nondietary intake of n-3 LCP to a placebo or control diet (17) reported that dietary and nondietary intake of n-3 LCP was associated with lower fatal myocardial infarction and sudden cardiac death in patients with CHD. However, n-3 LCP did not protect against nonfatal myocardial infarction, nonfatal CHD events, or total CAD morbidity (31, 63), suggesting perhaps that dietary fatty acid effects on hyperlipemic-induced atherosclerosis are distinct from those associated with arrhythmic myocardial dysfunction.

EPA and DHA are potent hypotriacylglycerolemic agents. Feeding 3 g/d of EPA + DHA to normolipemic subjects (91) or patients with elevated triglycerides (TGs) (92) reduced plasma TGs by 12% and 21%, respectively, compared to placebo. Harris (39) analyzed 36 well-controlled crossover studies in humans and found that consuming 3–4 g/d EPA + DHA resulted in a plasma TG decrease of 24% in normolipemic subjects and 34% in hypertriacylglycerolemic patients. EPA and DHA did not significantly alter plasma TC levels. However, LDL-C was significantly increased with ~3–6 g/d EPA + DHA intake in hypertriacylglycerolemic patients (22, 41). Plasma HDL-C levels were unchanged with EPA and DHA intake (39). EPA and DHA reduced plasma TG primarily by inhibiting hepatic TG and VLDL apoB secretion (40, 77). Recent mouse data indicate that EPA + DHA supplementation (at 5% en) as fish oil enhances hepatic beta-oxidation of fatty acids, thereby decreasing hepatic fatty acid availability for TG synthesis and secretion (59).

EPA and DHA are effective antiarrhythmic agents. Infusion of EPA + DHA in dogs made susceptible to fatal ventricular fibrillation and sudden cardiac death completely prevented ischemia-induced ventricular dysrhythmias (14, 15). In vitro induction of arrhythmias in cultured neonatal rat ventricular myocytes by pharmacological agents was abolished by the addition of EPA or DHA to the culture medium (54). A prospective, double blind, placebo-controlled study (94) found that a fish oil supplement providing 2.4 g/d (1% en) EPA + DHA for 16 weeks significantly reduced ventricular premature complexes in patients with frequent ventricular arrhythmia compared to the placebo group supplemented with sunflower oil. Furthermore, consumption of 4 g/d EPA + DHA increased heart rate variability in survivors of myocardial infarction (20), thereby reducing the risk of subsequent arrhythmic events. The antiarrhythmic action of EPA + DHA reflects the ability of the n-3 fatty acids to prevent calcium overload in cardiac myocytes during periods of stress (62).

The antiatherogenic action of EPA + DHA is partly due to effects on vascular function. Feeding 3 g/d of purified EPA or DHA significantly increased systemic arterial compliance compared to placebo in dyslipidemic subjects, indicating that these LCPs improve endothelial function (79). Further, in subjects with NIDDM, fish oil feeding for six weeks significantly improved large-artery compliance compared to olive oil feeding (69). A dose-dependent reduction in blood pressure has also been observed with EPA + DHA intake (75). A meta-analysis of controlled clinical trials showed that EPA + DHA intake of ≥ 3 g/d significantly lowered blood pressure (5, 75). Feeding 4 g/d of DHA significantly improved forearm blood flow and vascular reactivity compared to similar amounts of EPA or olive oil placebo in hyperlipidemic overweight men (74). Improved vascular function observed with EPA + DHA supplementation may be due to enhanced nitric acid production induced by EPA and DHA (39). Additionally, EPA and DHA are known to exert anti-inflammatory effects, some of which would be expected to deter atherogenesis. EPA + DHA intake (ranging from 0.3–2 g/d) produced a dose-dependent reduction in tumor necrosis factor alpha and interleukin-6 (103). EPA + DHA (~ 1 g/day) also reduced vascular adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and E-selectin (23, 102), which are implicated in plaque development. However, supplementation with increasing doses of EPA + DHA (0.5–2 g/d) did not alter mononuclear cell function or proinflammatory markers in healthy subjects (105). In hyperlipidemic and coronary patients, intake of higher doses of EPA + DHA (4.8–5.1 g/d) resulted in *increased* levels of vascular adhesion molecules (53, 93).

N-3 LCPs have also been reported to be antithrombotic. EPA competes with the arachidonic acid cascade that generates proaggregatory eicosanoids such as thromboxane B_2 . Increased fish consumption significantly reduced collagen-stimulated thromboxane B_2 production without altering prostacyclin synthesis in healthy men (64). EPA + DHA intake of 2 g/d (0.9% en) significantly reduced platelet aggregation in men at risk of CHD (73). N-3 LCP supplementation (4 g/d) also reduced coagulation factors such fibrinogen, Factor VII, and Willerbrand factor (21, 58, 95). However, other studies found no effects of EPA + DHA (0.9–4 g/d) on endogenous fibrinolysis, fibrinogen, Factor VII, or Willerbrand factor (6, 66). Thus, at clinically relevant supplemental intakes of EPA + DHA, major effects on thrombosis are not detectable and probably do not contribute appreciably to further their antiatherogenic effects.

A summary of effects of n-6 and n-3 fatty acids on cardiovascular risk factors across a wide range of intakes is presented in Table 1.

DIETARY n-6 AND n-3 PUFA INTAKE: MASS VERSUS RATIO

The above background data emphasize the need for both n-6 and n-3 PUFA in the diet, which follows from their both being essential fatty acids. Based on their combined impact on multiple systems, recommended intakes have been set for

TABLE 1 Biological effects of n-6 and n-3 polyunsaturated fatty acids on cardiovascular risk factors

Fatty acid	Parameters	Effect
Linoleic acid	Plasma lipids	
	TC	↓
	LDL-C	↓
	HDL-C	↑ or ↔ or ↓
	LDL-C:HDL-C	↓
	Hepatic LDL-C clearance	↑
	Hepatic LDL-C production	↓
Linolenic acid	Plasma lipids	
	LDL-C	↓ or ↔
	HDL-C	↓ or ↔
	LDL-C:HDL-C	↑ or ↔
	Arrhythmia	↓
	Thrombosis	↔ or ↓
EPA + DHA	Plasma lipids	
	TG	↓
	LDL-C	↑
	LDL-C:HDL-C	↑
	Hepatic TG and apo B secretion	↓
	Arrhythmia	↓
	Vascular endothelial function	Improved
	Blood pressure	↓
	Proinflammatory factors	↓ or ↔

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.

LA and LNA by the National Academy of Sciences (51). The adequate intake (AI) level for LA is 17 g/d for adult men and 12 g/d for women, based on diets with 20%–35% en from fat (51). This would correspond to approximately 6.4% en LA for men consuming 2400 kcal/d or women consuming 1700 kcal/d. Adequate intake for LNA is set at 1.6 g/d for men and 1.1 g/d for women, or 0.6% en LNA for adult men and women (51). In the United States, mean intake of LA is close to the AI and estimated to be 16 g/d for adult men and 11 g/d for women, based on the U.S. Department of Agriculture's Nationwide Food Consumption Survey (60). Mean LNA intake is estimated to be 1.5 g/d for men and 1.0 g/d for women (60). Adequate intake levels have not been established for EPA + DHA, which contribute ≤0.1% en (<0.2 g/d) in the typical U.S. diet (60).

ADEQUATE LINOLEIC ACID INTAKE Linoleic acid is the predominant dietary fatty acid regulating LDL-C metabolism. Because the hypercholesterolemic effects of SFA, trans fatty acids, and dietary cholesterol are all influenced by the available mass of LA (42, 43), an adequate intake of dietary LA in proportion to these

challenges is critical for preventing elevated plasma LDL-C, an established risk factor for CHD. In humans, high SFA intake (19%–30% en) significantly raised plasma TC and LDL-C when dietary LA contributed only 2.3%–4.5% en (4, 11, 67). In monkeys, the cholesterol-lowering effect of LA was nonlinear such that increasing dietary LA at low intakes of 1%–4% en resulted in major decreases in TC, whereas increasing LA at $\geq 5\%$ en intake had a less-pronounced cholesterol-lowering effect (42, 44, 86). The LDL-C-raising effects of 14:0 and 16:0 become progressively apparent as LA intake declines below 5% en (44, 85). Thus, maintaining LA intake above the threshold to counteract the SFA and dietary cholesterol insult is key to regulating LDL-C metabolism. It is to be emphasized that the LA threshold level for individuals may vary depending on other dietary and genetic factors. For instance, in overweight individuals with Metabolic Syndrome, hepatic LDL receptor activity would likely be depressed, thus requiring higher LA intake to counter this downregulation and thereby to lower plasma LDL-C.

Replacing LA with n-3 PUFA may not be effective for regulating plasma lipids, especially at high-SFA and low-LA intakes. In monkeys, replacing 4% en LA with a diet containing 1.4% en LA + 2.6% en LNA (all at 23% en SFA intake) significantly lowered HDL-C and raised the ratio of LDL-C to HDL-C (84). In humans, replacing 6.5% en LA with 2.5% en LA + 4% en LNA also tended to decrease HDL-C (55). Further, in moderately hypercholesterolemic subjects, the Mediterranean Diet enriched with LNA margarine (2.5% en LNA and 10% en LA) significantly lowered HDL-C and raised plasma TG compared to an LA-rich margarine (providing 11% en LA and 0.5% en LNA) (12). N-3 EPA + DHA intake at $> 1.0\%$ en can increase plasma LDL-C (39, 89). Replacing 6.5% en LA with 1.5% en EPA + DHA plus 5% en LA significantly raised LDL-C and the ratio of LDL-C to HDL-C in mildly hypercholesterolemic men (55).

Thus, taking into consideration the unique role of LA in lowering plasma TC and LDL-C and the nonlinear decrease in LDL-C elicited by LA, an intake of 5%–8% en from LA (11–20 g/d for a 2000-kcal diet) would represent adequate LA intake for healthy individuals. This is supported by epidemiological studies. In the National Heart, Lung, and Blood Institute Family Heart Study, 4.5%–6% en mean intake of LA was associated with significantly lower CAD compared to $< 3\%$ en mean LA intake (27). Salmeron et al. (90) found that women with mean LA intake of 7% en had a significantly lower risk of type 2 diabetes compared to those with $< 4\%$ en LA intake. Further, in well-controlled studies reporting beneficial effects of n-3 fatty acids for reducing the risk of CAD, LA contributed at least $> 4.5\%$ en (27, 50), indicating that adequate LA intake was maintained while n-3 intake increased. One expressed concern is that high LA intake might lead to increased AA synthesis, the precursor for proinflammatory and prothrombotic factors implicated in arterial plaque development. However, at moderate intakes of 5%–8% en, LA does not appear to adversely affect fibrinolytic factors, platelet aggregation, or inflammatory markers (32, 58, 105).

N-3 ADEQUATE INTAKE The n-3 fatty acids play a key role in improving vascular health and as antiarrhythmic agents. Epidemiological and randomized clinical trials show that intakes of 0.5%–1% en LNA are associated with significantly reduced risk of CHD compared to LNA intake <0.3% en (24, 27, 50, 99). Whether LNA is causally related is still less certain than the protection afforded by EPA + DHA. In a few studies demonstrating the beneficial effects of LNA on arterial compliance, arrhythmias, inflammation, and thrombosis, LNA contributed as much as 2%–5% en (33, 78, 87). However, the LNA effect on these factors at normal dietary intakes (<1% en) has not been rigorously investigated. Further, some studies have shown decreased plasma HDL and an increased ratio of LDL-C to HDL-C at LNA intake of >2% en (12, 84). Thus, LNA intake at approximately 0.75% en and possibly up to 1% en (2.2 g/d for a 2000-kcal diet) should adequately meet the cardiovascular needs of healthy adults.

Increasing evidence points to EPA and DHA as the most potent n-3 fatty acids influencing cardiac and vascular health. EPA and DHA intake of 0.2%–0.5% en (400–1000 mg/d) reduced the risk of CHD (18, 37, 49). EPA and DHA are the only dietary fatty acids consistently shown to lower plasma TG (39), an independent risk factor for CHD and type 2 diabetes mellitus. EPA + DHA supplements of 1%–1.8% en (2.2–4 g/d at 2000 kcal) led to significant reduction in plasma TG in normal and hyperlipemic subjects (39, 91, 92), and similar intakes reduced ventricular arrhythmias (20, 94), improved arterial compliance and endothelial function (79), reduced blood pressure (5, 75), and lowered proinflammatory markers (103), mostly in clinically affected subjects. Dose response effects of EPA + DHA, especially at lower doses of 0.5%–1% en, have not been studied adequately, especially for long periods (more than one year). However, most physiological effects have been apparent at 1% en EPA + DHA intake (92, 94, 103), which suggests that EPA + DHA effects may have their own threshold for effectiveness that plateaus between 0.5%–1% en intake (2, 49), possibly even lower if maintained for long periods. Also, EPA + DHA intake >1% en (2.2 g/d) has been associated with modestly increased plasma LDL-C (39), elevated blood glucose (34, 36), and increased VCAM-1, an atherogenic marker for cell adherence to endothelium (53, 93). In healthy individuals, ~5%–10% LNA is converted to EPA and ~2–5% to DHA (30, 35). Thus, 0.75% en LNA intake should contribute approximately 0.1% en as EPA + DHA. An additional EPA + DHA intake of 0.25%–0.5% en (500–1000 mg/d) should represent an adequate intake level for healthy adults.

FATTY ACID MASS VERSUS RATIOS The above discussion implies that the actual mass (as % en or g/d) of n-6 and n-3 fatty acids consumed in the diet, and not the ratio of n-6/n-3 PUFA, is the more important consideration for improving cardiovascular health. Realize, too, that the mass of 18:2n-6 required can vary, and depends, in part, on both dietary and genetic stressors affecting lipid (lipoprotein) metabolism. For instance, the International Society for the Study of Fatty Acids and Lipids workshop statement on the recommended dietary intakes for n-6 and n-3 fatty acids proposed 2%–3% en from LA, 1% en from LNA, and 0.3% en from

TABLE 2 Suggested adequate intake and recommended intakes of n-6 and n-3 polyunsaturated fatty acids versus current estimated consumption in the United States

	Linoleic acid (L.A) 18:2n6	Linolenic acid (LNA) 18:3n3	EPA + DHA 22:5n3; 22:6n3	n6:n3 ratio
National Academy of Sciences (adequate intake)				
(Reference 51)	6.4 % en	0.6% en	*	10:1
Men	(17 g/d @ 2400 kcal)	(1.6 g/d @ 2400 kcal)	*	
Women	(12 g/d @ 1700 kcal)	(1.1 g/d @ 1700 kcal)		
This review (recommendations)				
All adults	5–8% en ¹ (~15 g/d @ 2000 kcal)	0.75% en (~1.7 g/d @ 2000 kcal)	0.25% en (0.55 g/d @ 2000 kcal)	6:1
Current mean estimated intakes	6.3% en	0.75% en	≤0.1% en	12:1
(Reference 60)	(14 g/d @ 2000 kcal)	(1.7 g/d @ 2000 kcal)	(<0.20 g/d @ 2000 kcal)	

*No recommendation made.

¹Depends on other dietary fatty acids and individual lipoprotein status.

EPA + DHA, providing an n-6:n-3 ratio of roughly 2:1 (97). It also proposed an upper limit of LA at 3% en, which would be counterproductive in real terms for protecting against SFA and trans fatty acid elevations in LDL. Furthermore, intake of n-3 LCP beyond 1% en ordinarily offers minimal added benefits compared to intake of 0.25%–0.5% en EPA + DHA to the average person. Thus, at LA intake <4% en, hyperlipemic effects of SFA, trans fatty acids, and dietary cholesterol become more pronounced, and n-3 fatty acids at such low LA intake do not compensate for the LDL-C-raising effect of SFA. Further, neither epidemiological nor controlled clinical trials support a recommendation for limiting LA to <3% en as a means of reducing CHD risk. On the other hand, increasing n-3 PUFA intake (especially EPA and DHA) is consistently associated with lowered CHD risk. Thus, to confer optimal benefits with respect to CHD risk one should aim to increase n-3 fatty acids to 0.25%–0.5% en as EPA + DHA at the expense of SFA and MUFA, while maintaining LA intake at 5%–8% en.

Table 2 summarizes intake levels suggested for LA, LNA, and EPA + DHA.

SUMMARY

The n-3 and n-6 families of fatty acids are dietary essentials because they are required for normal physiological functions linked to membrane integrity and regulatory cell signals. The 18-carbon LA and LNA can be elongated and desaturated to LCP, including AA, EPA, and DHA. The balance of these n-6 and n-3 fatty acids in the diet is a critical factor influencing cardiovascular health. Relatively small amounts of these fatty acids are required in the diet to meet the needs of healthy adults. Deficiency of either n-6 or n-3 fatty acids is seldom encountered in typical Western diets. Based on the critical analysis of available literature, we suggest that an intake of about 6% en for LA, 0.75% en for LNA, and 0.25%–0.5% en for EPA + DHA would be adequate for most healthy adults. This corresponds to an n-6:n-3 ratio of ~6:1. However, intake of PUFA expressed in terms of mass (% en or g/d) is a more optimal approach to dietary n-6 and n-3 fatty acid balance than a simple ratio.

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