Chapter

Effects and Issues of Diet Fat on Cardiovascular Metabolism

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Abstract

Diet is a foundation of treatment for lifestyle-related diseases, such as high blood pressure, diabetes, and dyslipidemia. For these diseases, diet therapy has been disregarded in management of hyperlipidemia. Fat has more diversity of biological effects compared to those of protein and carbohydrate. New emerging evidences have resulted in a clear shift of recognition of fatty acids in diet therapy. The PREDIMED study has shown recently the amazing result that a calorie-unlimited, high-fat Mediterranean diet caused about 30% reduction in cardiovascular disease in obese subjects compared with a low-fat diet. Many authorities have removed restriction of intake of fat from their guidelines. The important, new message from recent medical and nutritional science is that people need to consume more "good fat" rather than limiting intake of fat to prevent cardiometabolic diseases. In this chapter, I would like to focus on the role of fatty acids with special relation on their effects on blood lipids and cardiovascular events.

Keywords: saturated fat, unsaturated fat, complex and refined carbohydrate, Mediterranean diet, olive oil, n-3 fish oil, primary and secondary prevention, LDL cholesterol, antioxidant, diet guidelines

1. Introduction

Diet therapy has been disregarded in management of hyperlipidemia. Statistical data for the US between 1990 and 2016 show that in addition to tobacco consumption, poor diet and subsequent obesity are one of the major reasons for mortality [1]. Generally, information about the benefits of nutritional interventions has not adequately been translated into action in medical training or practice [2]. In a 2017 online survey of 646 cardiologists in the US [3], 90% reported that they had not received adequate nutrition education to be able to counsel their patients, even though 95% believed it was their personal responsibility to do so.

Compared with pharmacological trials, high-level evidence about diet therapy is limited. Among the three macronutrients, data around fat have been especially controversial. This is partially due to the more diverse roles of fat compared to protein or carbohydrate. Fat is not only a source of energy production but also a major component of hormones, and cell and nuclear membranes, and a carrier for the fat-soluble vitamins. Furthermore, essential fatty acids are involved in many physiological processes such as inflammation, cell proliferation, wound healing, and blood coagulation. The data about diet therapy are frequently inconsistent even for apparently solid recommendations in authorized guidelines. In this chapter, I would

like to focus on the role of fatty acids with special relation on their effects on blood lipids and cardiovascular events.

2. Incidence of formal diet consultation in Japan

In Japan, formal professional dietary intervention is not so common. Generally, registered dietitians provide recommendations for meals according to the patients' disease conditions from medical, nutritional, and hygienic aspects. Statistical data from the two university hospitals in Tokyo indicate that the number of dietary referrals from physicians for patients with dyslipidemia is less than 5% of total cases, which is in sharp contrast to those with diabetes at more than 50% [4, 5]. There are several reasons to explain the fewer consultations, including scanty data in diet intervention trials in Japanese population, weak recognition of effectiveness of diet therapy for dyslipidemia among physicians, and most importantly, patients find it easier to take statins than follow diet therapy. Compared with LDL cholesterol reduction by diet therapy, statins are more powerful, with up to 50% reduction in LDL cholesterol. Results in the PREDIMED study have clearly shown that a Mediterranean diet enriched with extra-virgin olive oil or nuts reduced CV events by 30% [6]. This magnitude of CV event reduction was compatible with those of statin trials, and importantly, it was achieved with small changes in LDL cholesterol, blood pressure, and blood glucose. The data in the PREDIMED study have clearly shed light on distinctive features of the power of diet therapy, which affects many aspects of not only classical risk factors but also other unknown biological processes modulating the pathophysiology of diseases.

3. Effects of dietary components on blood lipid

Major diet factors that affect blood LDL cholesterol include calories, three major nutrients (protein, fat, and carbohydrate), fat type, fiber, food sterol, etc. By modifying these factors, a Mediterranean diet and DASH diet are intended to improve cardiometabolic risk factors. These two diets ranked as top diets according to a US News and World report in 2018. They share many aspects beneficial for maintaining health. Especially, intake of healthy fats such as olive oil rather than saturated fatty acids (SFA) is a mainstay in the Mediterranean diet. The basic principle in diet is not to eat too much or too little of one component of foods, but rather to eat good balance of foods. Trials that change a single dietary factor have an advantage that makes them suitable to clarify the contribution of some specific component on diet parameters. Studies to evaluate effects of SFA or salt on LDL cholesterol or blood pressure are good examples. By contrast, the degree of efficacy and durability are, generally, greater in trials changing the entire diet style. Thus, this type of intervention trial, such as Mediterranean vs. typical western-style diet, is more suitable as a hard endpoint study.

3.1 Calories and plasma lipids

Optimization of intake of total calories is a foundation of maintenance of healthy life. Reducing food intake to induce undernutrition extends the life spans of multiple species, ranging from single-celled organisms to mammals [7]. By contrast, whether calorie restriction decreases CV events in human remains unclear except in bariatric surgery. The LookAHEAD study examined whether an intensive lifestyle intervention for weight loss for 10 years would decrease CV morbidity and

mortality among 5145 obese patients with type 2 diabetes [8]. The intensive lifestyle intervention was aimed at achieving and maintaining weight loss of at least 7% by focusing on reduced caloric intake (calorie goal of 1200–1800 kcal per day, restricting fat calories to <30%) and increased physical activity. Although greater reductions in all CV risk factors was observed in the intervention group than in the control group, the rate of CV mortality and myocardial infarction was not different (**Figure 1a**). Rebound of weight in the intervention group (**Figure 1b**) increased the statin use in the control group, and the lack of instruction about saturated and unsaturated fats is suggested for reasons of the negative results in this study. Five years later, in the PREDIMED study [6], a Mediterranean diet loaded with high content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUSA) without calorie restriction and exercise recommendation reduced CV events significantly approximately by 30% compared to the control low-fat diet group (**Figure 2**). It is noteworthy that the reduction of CV events occurred in the Mediterranean diet groups that consumed about 200–250 kcal higher calories as fat than in the control group. The data of these two studies [6, 8] have strongly suggested that intake of unsaturated fat is more effective than a low-fat, low-calorie diet for reduction of CV events. However, weight reduction by the low-fat diet in the LookAHEAD study has brought in important health benefits other than CV event reduction. Patients in the low-fat group were more likely to have a partial remission of diabetes during the first 4 years of the trial [9], more improvement in terms of reductions in urinary incontinence [10], sleep apnea [11], and depression [12] and improvements in quality of life [12], physical functioning [13], and mobility [14] than were those in the control group. Calorie restriction by diet is effective in reducing body weight in obese subjects. Therefore, diet therapy should be individualized dependent on the treatment goal, patient's characteristics such as underlying disease, BMI, and daily diet habits.

The difference in LDL cholesterol in the two groups in the LookAHEAD study was 1 mg/dl at the end of study [8]. By contrast, the effects of bariatric surgery on plasma LDL cholesterol and CV events have been clearer, because bariatric surgery reduces body weight to a greater extent, 20–40% from baseline. In a study of registry data in Sweden, LDL cholesterol was reduced approximately 40% associated with a 30% decrease in all-cause mortality 15 years after the surgery [15].

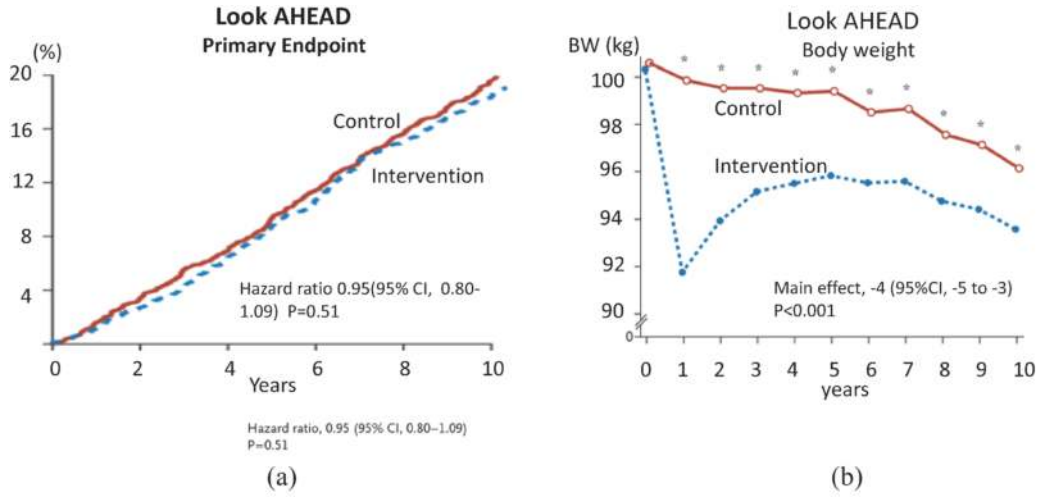

Figure 1.
(a) Kaplan-Meier curves of the primary end point in look AHEAD study [8]. The primary outcome was a composite of death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for angina. (b) Changes in body weight in the look AHEAD study [8].

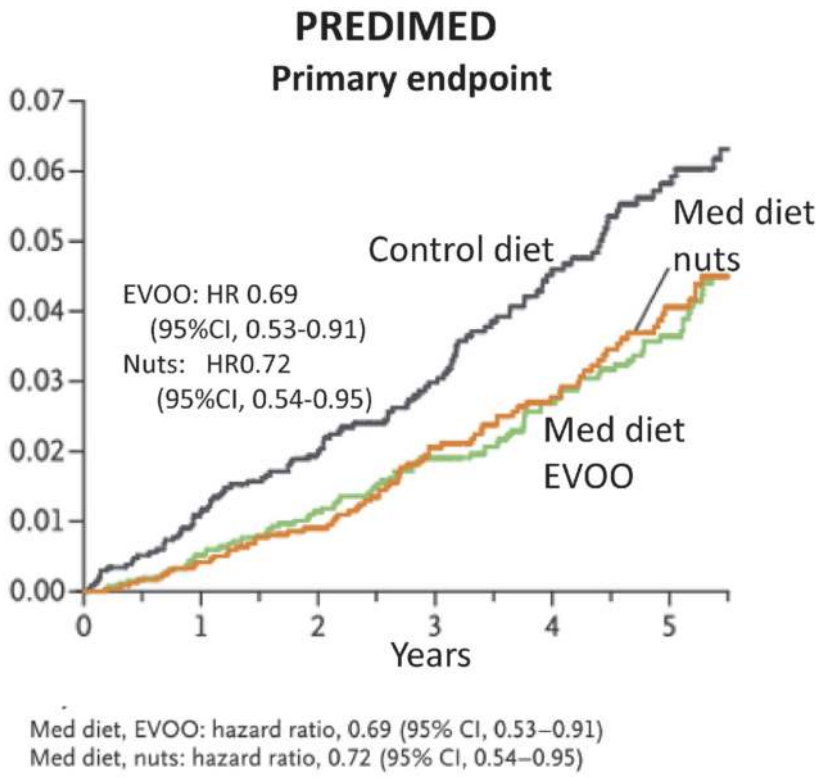

Figure 2.
Kaplan-Meier curves of the primary end point in the PREDIMED study [6]. The primary end point is a composite of acute myocardial infarction, stroke, and death from cardiovascular causes.

Bariatric surgery has also resulted in the remission of concomitant risk factors. A US cohort study in 2458 obese subjects showed that after gastric bypass surgery, the remission rate of was 62% for dyslipidemia, 38% for hypertension, and 68% for diabetes [16]. More than 150,000 bariatric surgeries had been performed in the US in 2013, which were almost one third of operations performed globally in 2013 [17]. Data from the bariatric surgery clearly indicate that it is important to provide substantial and sustainable body weight reduction for obese subjects in order to decrease CV events. If patients have difficulty maintaining calorie restriction, emerging evidence has been accumulating that a calorie-unlimited but well-balanced diet with MUFA/PUFA is a good choice.

3.2 Data and issues of low-carbohydrate vs. low-fat diet

In order to prevent muscle and bone wasting in aged people with sarcopenia, many scientific guidelines recommend that protein intake should be at least 1 g/kg/day of standard body weight unless renal function is abnormal. Thus, in limiting dietary calories, the requirement for protein usually cannot be changed. Much controversial data have been reported regarding whether the restriction of carbohydrate or fat is better in subjects with obesity or diabetes. Reduced carbohydrate diets are defined as having carbohydrate intake below the Dietary Guidelines for Americans (DGA) recommendation (45–65% of total energy intake). Regarding effects on body weight, several previous systematic reviews and meta-analyses [18–20] have shown that low-carbohydrate high-fat diets are just as effective, if not more so, than low-fat high-carbohydrate diets. Excess energy from carbohydrates stimulates the induction of lipogenesis in the liver via SREBP-1, resulting in accumulation of triglycerides (TG) in many organs [21], which induces obesity,

dyslipidemia, and insulin resistance. Thus, the restriction of carbohydrates results in not only weight reduction but also improvement of dyslipidemia and insulin resistance.

Evidence has shown that under the controlled condition, both of the total calorie and the type of carbohydrate can affect body weight positively or negatively and plasma lipid profile as well. Even when receiving a high-calorie diet with increased fat content, obese subjects (BMI > 30) could reduce more weight with a low refined carbohydrate diet, compared with high-refined carbohydrate, low-fat, and energy-restricted diets in 1 year, based on a meta-analysis [22] and a 2-year intervention study [23]. One general consensus is that because refined carbohydrates are associated with high glycemic index, limiting intake of refined carbohydrates improves postprandial hyperglycemia, resulting in lower insulin release as well as decrease in body weight. In the period 1980–1990, calorie restriction by eating low-fat food was strongly recommended in many places in the US. The big issue of this nation-wide trend of low-fat diet was that most people were eating food containing refined carbohydrates with high glycemic index instead of fatty food (**Figure 3a**). An obesity epidemic in this period in the US coincided with the low-fat campaign. During the epidemic, the rate of obesity was almost triple, and the rate of diabetes doubled compared with three decades earlier [24, 25] (**Figure 3b**). It is ironic that people lost more money (low-fat food was generally more expensive) and gained weight while eating a low-fat diet.

In the OmniHeart study [26], the recommendation of fructose-sweetened beverages defined in the protocol could be one reason why the high-carbohydrate diets group showed the worst cardiometablic effects. Because of recent data showing fructose has worse effects on cardiometabolic risk markers than any other carbohydrates, the conclusion from the OmniHeart study cannot be extrapolated to all carbohydrates. Beverage makers in the US used to actively campaign by insisting that "over calories" was the reason for obesity rather than the type of sweetened carbohydrates included in beverages. However, each mono- and disaccharide has different effects on body metabolism. In a feeding study in overweight subjects, comparison of drinking glucose- or fructose-sweetened beverages for 10 weeks showed that after fructose intake, fasting plasma glucose, insulin levels, and visceral adipose volume increased with a decrease in insulin sensitivity compared with the intake of glucose-sweetened beverages [27]. Glucose metabolism in blood and liver is tightly regulated by insulin and glucagon. On the other hand, because fructokinase does not play any role in the regulation of accumulation of fructose in the liver, the concentration of fructose in blood and liver increases with oral intake of fructose, which is called unregulated fructose uptake [28, 29]. Consequently,

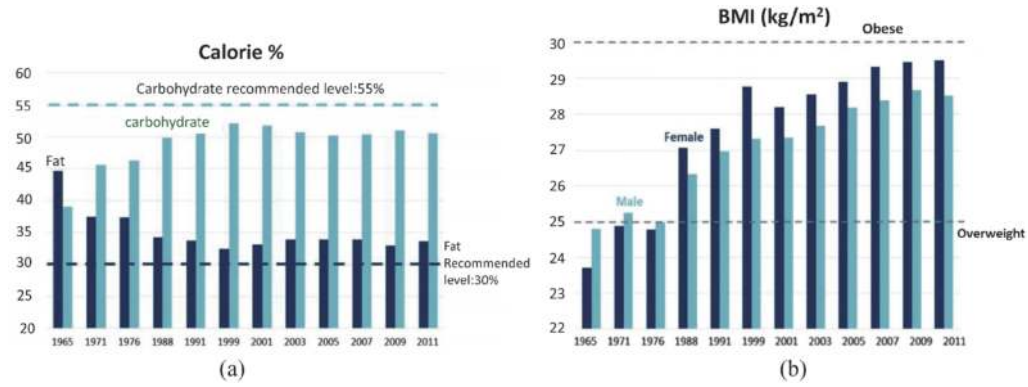

Figure 3.
(a) Changes in average fat and carbohydrate caloric consumption in adults from 1965 to 2011 [25].
(b) Changes in average BMI in adults from 1965 to 2011 [25].

accumulated fructose in the liver stimulates de novo lipogenesis and results in detrimental effects on glucose and lipoprotein metabolism [27].

Because of few reliable RCT, there have been many controversial results when comparing beneficial effects between low-fat and low-carbohydrate diet. It appears that the type of macronutrients replaced for fat or carbohydrate has significant effects on those results. A recent meta-analysis of studies with 432,179 subjects has shown that participants in low (<40%) and high (>70%) carbohydrate consumption groups had greater mortality than in moderate consumption groups, which is consistent with a U-shaped association [30]. The results varied according to the source of macronutrients. Namely, the mortality increased when carbohydrate was replaced with animal-derived fat or protein, and the mortality decreased when the substitutions were plant-based. This indicates that food source can be an important consideration for CV outcomes when one macronutrient is replaced with another macronutrient. It has been hypothesized that diets of lower plant carbohydrate with increased animal protein and fat stimulate inflammation, aging, and oxidative stress. On the contrary, nutrition based on low-carbohydrate and high-fat diet may have anti-inflammatory, anti-oxidative, and anticancer effects. A diet that contains reduced carbohydrate with higher fat or even a ketogenic diet, very low carbohydrate diet (<10% of carbohydrate calorie), slows down cancer growth and proliferation [31, 32]. Before instructing a patient to follow a low-fat or low-carbohydrate diet, clinical factors should be considered, including age, body weight, diet habits, underlying diseases, and kidney function. For example, a 75 y/o obese subject with diabetes who consumes a high-calorie diet with high content of fat from animal origin should decrease animal fat intake to reduce total calories for control of body weight. It makes sense to replace animal-fat with complex carbohydrates rather than plant-based unsaturated fat. This way would protect against the increase in total calories and let the glucose level decrease. If a subject has a history of myocardial infarction, partial substitution of animal fats with MUFA, such as found in olive oil, is another option, because of protection of CV disease by intake of olive oil. By considering many aspects of benefit and harm of carbohydrates and fat in this way, appropriate dietary composition should be individually fine-tuned based on patients' clinical characteristics and treatment goals.

3.3 Comparison of guidelines in Japan, the US, and Europe regarding fat content

Table 1 summarizes comparison of recommendations about dietary fat in several key scientific societies in the US, Europe, and Japan. Calorie intake from fat is restricted to 20–30% in guidelines of Ministry of Health, Labour and Welfare (MHLW) of Japan [33], Japanese Diabetes Societies (JDS) [34], and Atherosclerosis Societies (JAS) [35]. This value of 30% is classified as low-fat diet as defined by the 2014 the American Diabetes Association (ADA) guideline [36]. There are several reasons why restriction of calories from fat still remains in these recommendations in Japan. First, the LDL cholesterol level in Japanese is steadily increasing recently and at present it is higher among Japanese females than in American females [37]. A high-fat diet generally increases intake of SFA, resulting in increases in LDL cholesterol. Second, BMI in Japanese people is lower than in Caucasians. It is well known that thin Asian people are prone to develop diabetes with a mild increase in body weight. Therefore, because increases in fat calories are usually associated with increases in total calories and subsequently in body weight, restricting fat calories is often helpful to prevent worsening in blood glucose in diabetic subjects.

	Atherosclerosis/dyslipidemia			Diabetes			
	JAS (2017)	**AHA/ACC (2013)**	**ESC/EAS (2016)**	**JDS (2016)**	**ADA (2014)**	**AACE (2013)**	**USDA (2015)**
Total fat	20–25%	N/A[a]	N/R[b]	20–30%	N/R[c]	25–35%	N/R
MUFA	N/A[d]	N/A[d]	N/A	N/A	N/A[e]	<10%	N/A[d]
PUFA	N/A[d]	N/A[d]	<10%[e]	N/A	N/A	<20%	N/A[d]
Fish (n-3)	↑[f]	>Two times/week	↑[g]	↑[h]	>Two times/week	>Two times/week	>8 oz/ week
SFA	4.5–7%	<5–6%	<7%	<7%	<10%	<7%	<10%
Trans fat	<1%	↓[i]	<1%	↓[i]	↓[i]	<1%	↓[i]
Cholesterol	<200 mg	N/A[j]	<300 mg	<300 mg	<300 mg	<200 mg	N/A[j]

N/A: not available; N/R: no restriction; MUFA: monounsaturated fatty acids; and PUFA: polyunsaturated fatty acids.
[a]Recommendation for Mediterranean diet which usually has 32–35% as fat calorie.
[b]No restriction of fat intake, which is dependent upon individual preferences. However, fat intakes at >35% of calories are generally associated with increased intakes of both saturated fat and calories.
[c]No restriction of fat intake, inconclusive for an ideal amount of total fat intake for people with diabetes; therefore, goals should be individualized. Fat quality appears to be far more important than quantity.
[d]Not stated on intake of mono- and polyunsaturated fat. However, there are comments of improvement of LDL cholesterol by substitution of SFA with MUSA or PUFA.
[e]Intake of n-6 PUFAs should be limited to 10% of the energy intake in order to minimize the risk of lipid peroxidation of plasma lipoproteins and to avoid any clinically relevant HDL-C decrease.
[f]Recommend intake of fish. Increase in n-3 fat decreases plasma TG level with potential beneficial effects on CV events.
[g]Recommend intake of fish. Increase in n-3 fat decreases plasma TG level. Unknown for reduction of CV events.
[h]Recommend intake of fish. However, increase n-3 fat has no effects on prevention of diabetes and on CV events in diabetes.
[i]No upper limit defined. However, should decrease as much as possible.
[j]No upper limit.

Table 1.
Recommendations for intake of fat and cholesterol-related nutrients in major guidelines.

By contrast, in of the 2014 ADA guideline [36], lifestyle management by American Heart Association (AHA)/the American College of Cardiology (ACC, 2013) [38], European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS, 2016) [39], and the Dietary Guidelines for Americans 2015–2020 [40], the upper limit in fat calories has been actually dropped, and they describe that it is advisable to determine it on an individual basis along with maintaining whole calories. Each of these guidelines stresses quality of fat rather than the total quantity of fat intake. There are a few important considerations for this major change. First, many people tend to often eat more refined carbohydrates as replacement for fat, and previous data have not shown clearly that this would reduce CV mortality and motility. A high-fat diet reduces body weight more than a high-refined carbohydrate diet [22, 23], which favors abandoning the upper limit of total fat intake from many guidelines. Second, limiting overall fat intake habitually carries the potential risk of reducing consumption of "good fat" such as MUFA/PUFA [36, 38–40]. All the guidelines have quoted data in the PREDIMED study, where a calorie-unlimited, fat-unrestricted Mediterranean diet reduced CV events about 30% compared with a low-fat diet [6]. It is noteworthy that fat calories in the Mediterranean intervention group was exceeded 40% in the PREDIMED study. The important message in removing the upper limit for fat calories from these guidelines is that to maintain consumption of an appropriate level of calories, one must include healthy high-quality fat in the diet.

3.4 Issues and limitations around nutritional trials

There have been several limitations of studies in nutritional science. Usually, a nutritional study lacks a double-blind design and a placebo for food. Without a placebo-controlled design, the study can provide no true evidence of either benefit or harm of the intervention. For example, if SFA were replaced by carbohydrate or protein, the isolated effect of SFA would be influenced by the changes in the composition of the other macronutrients to keep the diet calorie constant, which would make the isolated effect of SFA difficult to evaluate. Even in the subcategories of SFA, MUFA, and n-3, n-6 PUFA, there exists clear heterogeneity within each group that contributes to different biological effects. Furthermore, the food content within its SFA can have a significant impact on CV risk. The fermentation of dairy products provides a good example. The content of SFA in cheese is very high (20 g in 100 g of natural cheese compared with 3 g in 100 g of beef sirloin). A 10-year cohort study showed that the consumption of SFA from dairy products was associated with decreased risk of CV disease, and, by contrast, a higher intake of SFA from meat (including red and processed meat and poultry) was associated with greater CV disease risk [41].

Under- and over-reporting of dietary intake is common in nutritional studies. In contrast to easily countable items like coffee and sweetened beverages, the percentages of energy from fat and added sugar based on a food frequency questionnaire (FFQ) were underestimated [42]. Regarding longevity of habits of food intake, the protocol design in one cohort study had a diet assessment at baseline and approximately 36,000 participants were followed for 12 years [43]. This is a common type of study, in which a one time-point assessment can predict the occurrence of disease many years later.

The logic behind limiting the intake of SFA is derived from the risk factor model for coronary heart disease (CHD) causality, in which LDL cholesterol is a causal factor for CHD and a diet including SFA increases LDL cholesterol compared with other macronutrients. However, other biomarkers that predict CHD risk more have been proposed such as total cholesterol to HDL cholesterol ratio, small dense LDL cholesterol, apolipoprotein B, and others. Therefore, total CHD risk may be increased with elevations of these biomarkers even if LDL cholesterol does not change after the diet intervention.

Evidence coming from RCT is generally positioned above evidence from cohort studies in the research hierarchy. Findings in diet- and health-related RCTs are not necessarily more reliable than those from well-conducted cohort studies [44]. RCTs sometimes include subjects with underlying diseases and follow them up with a relatively short period. This means substantial limitation to extrapolate findings from RCTs to healthy persons, when CV events occur rarely over many years. Cohort studies also have their own sources of error, especially issues of confounding factors. For example, SFA intake is associated with behaviors indicating lower health consciousness, [45] whereas PUFA intake is either associated with behaviors indicating greater health consciousness [46], or is not related to health consciousness [47]. Therefore, confounding variables also often explain the inconsistent results regarding the replacement of SFA with PUFA for prevention of coronary heart disease. When reading manuscripts in nutritional studies, one must keep in mind these limitations and issues included in the studies.

3.5 Effects of replacement of SFA with PUFA

A high-SFA diet is quite palatable with weak effect on satiation, which results in potentially overconsumption and obesity [48]. There has been a tremendous amount

of data showing that reducing SFA in food resulted in health benefit by lowering blood LDL cholesterol and CV events in RCT [49, 50], prospective cohort studies [51–53], and epidemiological studies [54]. Based on this evidence, AHA has recommended to decrease over-consumption of SFA since 1961. On the other hand, one RCT [55], a prospective cohort study [43], and meta-analysis [56] have reported that increases in SFA are not associated with increases in CV events. In the US where both myocardial infarction and consumption of beef are more than triple than that in Japan, whether the consumption of SFA in food results in health problems should be a serious issue [57, 58]. One recent article in the Annals of Internal Medicine in 2019 has concluded that red meat may have little or no effect on CV outcomes and cancer mortality based on low- to very-low-certainty evidence [59]. Just after this publication, roughly 2000 emails, mostly caustic in tone, were sent to the inbox of an editor in the journal as a wave of backlash, including a push from one group attempting to have the guideline retracted even prior to publication.

The message from guidelines in major scientific societies reaches the same conclusion that limiting intake of SFA by replacing with MUFA and PUFA reduces LDL cholesterol and potentially reduces CV diseases. This conclusion was summarized in a position paper by the 2017 AHA presidential advisory board [60], which relied on four core randomized trials [61–64]. Results of a meta-analysis of these four core trials [60] are shown in **Figure 4**. The results showed that lowering calorie from SFA down to 7% and replacing them with vegetable oil rich in PUFA, primarily soybean oil, lowered blood cholesterol by 15% and the incidence of coronary heart disease (CHD) by 29%. The degree of efficacy by cutting this amount of SFA is compatible with that of statins [65].

Although it has become now widely accepted that the dominant dietary factor involving coronary heart disease is an excessive intake of SFA, recent new evidence has suggested that SFA may play a much less important role in coronary heart disease than was previously believed. As discussed previously, potential biases have been often included in diet studies, and earlier meta-analyses did not sufficiently account for major confounding variables. One study has shown different results in two meta-analyses of collecting only adequately controlled trials from collecting only inadequately controlled trials (**Figure 5**) [66]. These data clearly represent one typical example of difficulty in conducting well-controlled diet trials. The manuscript of the 2017 AHA presidential advisory board [60] that selected the 4 core RCTs may have had potential bias by excluding or including some studies from the

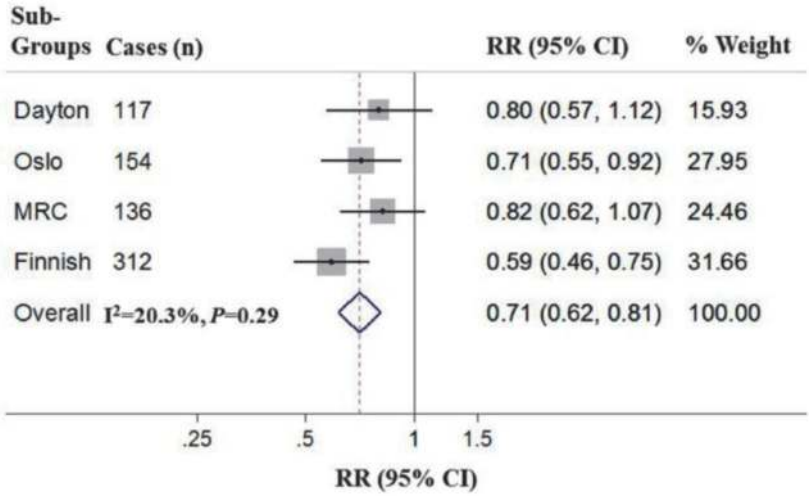

Figure 4.
Meta-analysis of core trials on replacing saturated with polyunsaturated fat [60].

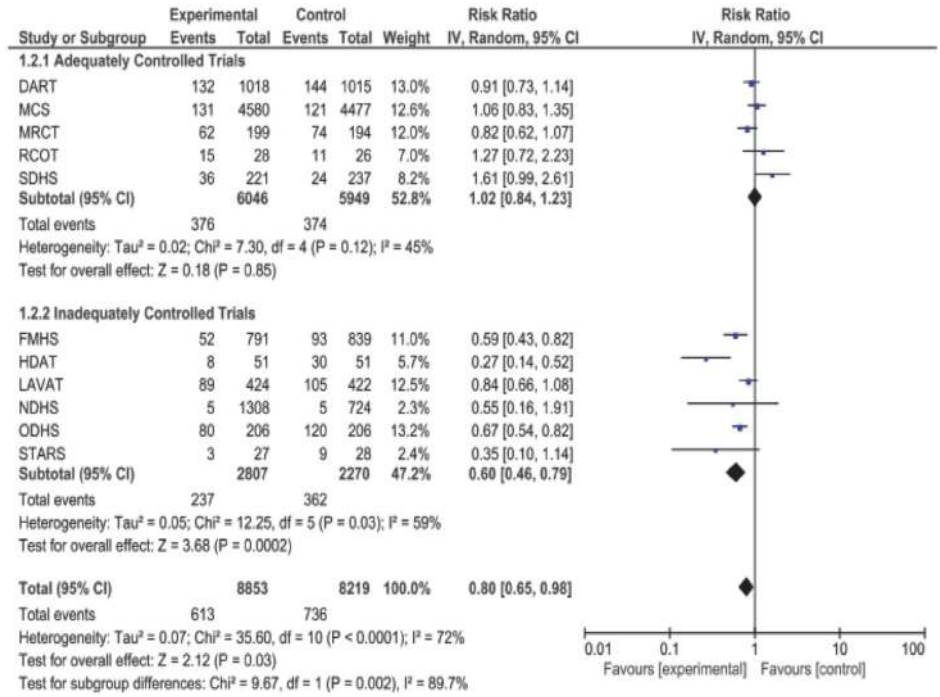

Figure 5.
Forest plot showing pooled RR with 95% CI for the number of total CHD events [66].

analysis. Notwithstanding apparently consistency of the description in many authoritative guidelines of harmful effects of SFA, some experts are insisting that there have been no solid evidence indicating that the consumption of SFA is related with higher incidence of CV disease [67].

It seems that one common key message in studies that the overestimated harmful effects of SFA is that other factors (e.g., increased refined carbohydrate/added sugar included in soft drinks, or trans fat; or decreased fish, fruit, and vegetables) play a more role in development of coronary heart disease than SFA [68]. However, previous data of substitution of SFA with PUFA have consistently shown improved blood lipid profile and blood pressure. Furthermore, increased intake of SFA from animal meat and butter generally is accompanied by more consumption of trans fat or other substances with potentially harmful effects on humans. The Mediterranean and DASH diets, which include high amount of MUFA and PUFA with low contents of SFA, have constantly shown health benefits. Based on all these data, as in the authorized opinions, restriction of SFA is a reasonable strategy.

3.6 Effects of replacement of SFA with MUFA

Compared with PUFA, less data have been available evaluating benefits of MUFA [69]. In human cohort studies, the replacement of SFA with MUFA yielded smaller reduction of LDL cholesterol [70] and incidence of CHD [71–73], compared with PUFA. One huge cohort study in 127,536 subjects combining the Nurses' Health Study and the Health Professionals Follow-up Study has shown that replacing 5% of energy intake from SFAs with equivalent energy intake from PUFA or MUFA was associated with a 25 and 15% lower risk of CHD, respectively (**Figure 6**) [73]. A review of the Cochran database in 2015 has concluded that replacing the energy from SFA with PUFA appears to be a useful strategy and that, by contrast, effects of replacement with MUFA were unclear due to inclusion of only one small trial [69].

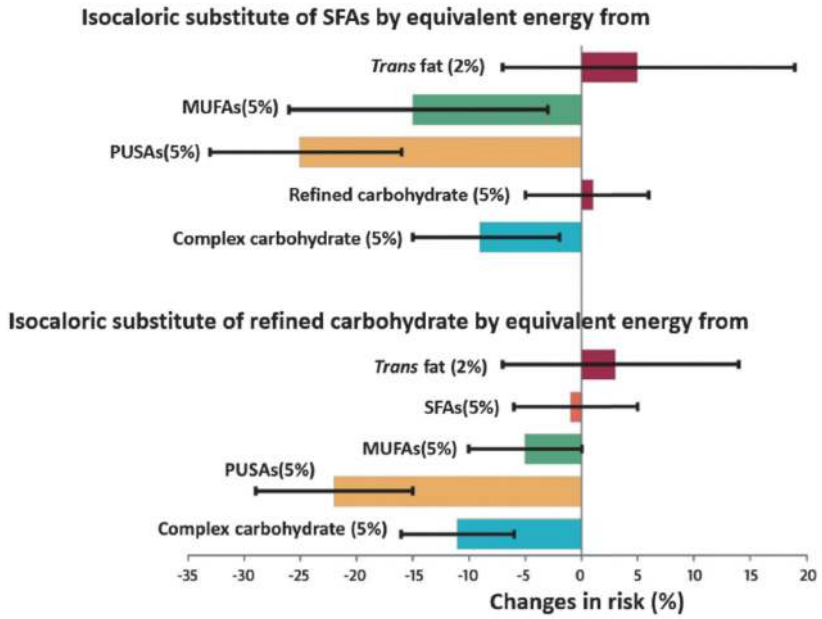

Figure 6.
Estimated percent change in the risk of CHD after isocaloric substitute of SFA (upper half) or refined carbohydrate (lower half) by other nutrients [73].

The source and origin of MUFA within a specific diet may explain the inconsistent previous results with MUFA [74]. One huge meta-analysis including 32 cohort studies in 840,000 subjects has shown data that effects of MUFA on CV disease and its mortality varied depending on the different dietary sources of MUFA. Whereas MUFA of mixed animal and vegetable sources per se did not yield any significant effects on major CV outcomes, importantly, significant associations could only be found between higher intake of olive oil and reduced risk of CV events. It is of note that all studies which showed benefit for CV risk were conducted in the Mediterranean nations where extra-virgin olive oil is the most dominant source of this type of fatty acid [75]. The amounts of olive oil in the highest consumption group used in those studies were > 30 g/day in the EPIC cohort (in Spain), 48 g/day in the EPIC cohort in Greece [76], 52 g/day in a case-controlled study in Spain [77], and 56 g/day in the PREDIMED study [78]. Although mean olive oil consumption in the US is increasing, it is still very low at 4.2 g/day in 2010 compared with those in the Mediterranean areas (**Figure 7**) [79]. Another study in Spain has shown clearly different effects on cardiometabolic parameters between olive oil and sunflower oil (11% for MUFA and 60% for PUFA) [80]. With a slight increase in LDL cholesterol in the olive oil group, olive oil improved other risk factors such as glucose, TG, and body weight to a greater extent than in the sunflower oil group. The amount of olive oil in this study was expected to be more than 40 g/day, based on data showing that Spanish diet usually includes olive oil accounting for 18% of calorie intake [81]. All of these findings indicate that a large amount of olive oil (probably > 30 g/day) has distinctive effects compared with other oils from plant or animal origin.

Along with firm evidence of CV event reduction in two RCTs (Lyon Diet Heart study [82, 83] and PREDIMED [6]), olive oil should be viewed not only as "better" MUFA but also as a nutrient including many biologically active ingredients such as polyphenol. In 2018, the FDA approved a qualified health claim for consuming oils with high levels of oleic acid (major MUFA in olive oils) to reduce risk for coronary heart disease [84]. This new claim allows manufactures of olive oils to state that "supportive but not conclusive scientific evidence" suggest that daily consumption of one and half tablespoon (20 g) of olive oil may reduce CHD risk.

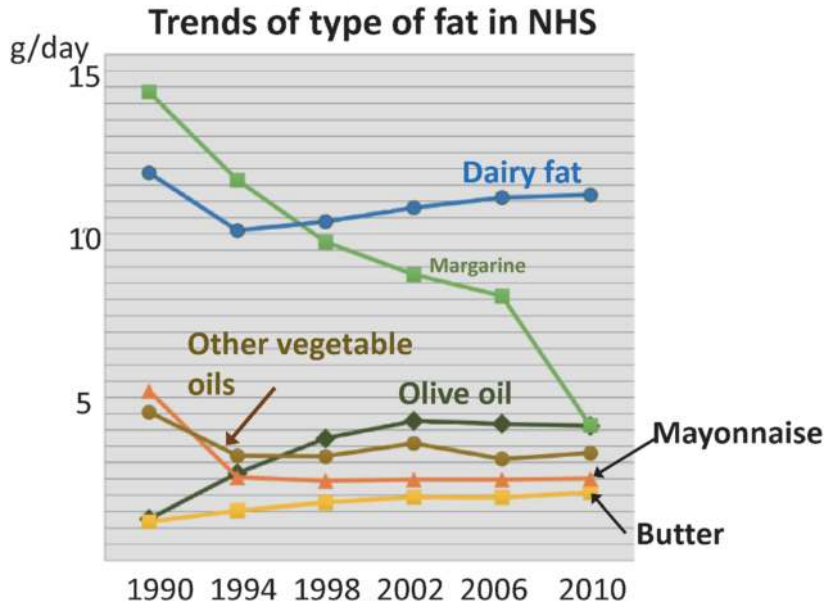

Figure 7.
Trends of types of fat intake from 1990 to 2010 in Nurses' Health Study (NHS) [79].

3.7 Effects of replacement of saturated fat with carbohydrate

In many studies which replaced SFA with carbohydrate, total calories from fat was roughly reduced from 40% to 20–30%, and this has affected diet adherence in subjects who are accustomed to eating oily food. In the Women's Health Initiative Study [85], the protocol intended originally to reduce fat calories from 37 to 20% in the low-fat/high carbohydrate group. In 8 years, the fat calories increased to 37% in this group, which was higher than the fat calories in the control group (35%) because of poor compliance. In the low-fat/high carbohydrate group, although LDL cholesterol decreased by 3 md/dl, CV events were not reduced significantly. One meta-analysis including 15 randomized controlled trials with 59,000 participants [69] concluded that the replacement of SFA with carbohydrates reduced LDL cholesterol mildly (5%) without affecting CV events. In most of the studies used in this meta-analysis, it is noteworthy that refined carbohydrates were incorporated as replacement for SFA. A growing weight of authoritative opinion is to recommend intake of complex carbohydrates including much fiber and minerals. Two recent studies [56, 86] have also shown the same results that the CV benefit by reduced SFA tends to be neutralized by increasing intake of refined carbohydrates.

Combined analysis of two prospective, cohort studies [73] in the Nurses' Health Study (84,628 women) and the Health Professionals Follow-up Study (42,908 men) has the advantage of investigating effects of different sources of carbohydrates, refined or complex, on CHD risk for long periods up to 30 years. **Figure 6** shows that when 5% of energy from SFA was replaced with 5% of energy from carbohydrates, the risk of CHD decreased by 9%. By contrast, when replaced with refined carbohydrates/added sugars, the risk of CHD did not decrease. This study has also shown change of CHD risk when refined carbohydrates/added sugars were replaced with other nutrients. Replacing refined carbohydrates/added sugars with MUFA, PUFA, or complex carbohydrates was significantly associated with a lower risk of CHD (5, 21, and 12%, respectively). However, there was no change in the risk of CHD when refined carbohydrates/added sugars were replaced with SFA. Results of this cohort study provide additional evidence that effects of refined carbohydrates on CV risk are at least equipotent to those of SFA.

At present, intact or minimally processed carbohydrates where fiber, bran, and germ content is high are generally called complex carbohydrates. Bran of whole grain is fiber-filled outer layer with many vitamins and minerals, and germ, which is a nutrient-packed core, includes phytochemicals, unsaturated fat as well as vitamins. Differences in complex and refined (simple) carbohydrates are shown in **Table 2**. The mechanisms for harmful cardiometabolic effects of refined carbohydrates are mostly explained by their ability to increase insulin release and shorten satiety [87]. Refined carbohydrate also do not have as much vitamins and fibers as complex carbohydrates. A diet with refined carbohydrates with high glycemic index such as rice is associated with higher incidence of coronary heart disease and diabetes [88–91]. Reduction of refined carbohydrates in food has decreased blood pressure, TG, and high-sensitivity CRP, and improved insulin resistance [92, 93].

Emerging evidence regarding the harmful effects of SFA and refined carbohydrates on cardiometabolic markers has exerted significant effects on the food label authorized by FDA. Some experts have opinion to recommend on leaving percent daily value of SFA with dropping % total fat, and adding on % refined carbohydrate instead of % carbohydrate against the daily value of these nutrients [94]. Because of data showing that refined carbohydrates with high glycemic indices worsen diabetic status, the FDA changed the nutrition facts section of the food label in 2019 to require listing the amount of and percent daily value for added sugars (**Figure 8**, arrow 1). Furthermore, "Calories from Fat" was removed, because abundant data show that the type of fat is more important than the amount (**Figure 8**, arrow 2).

Foods and drinks that contain no significant nutrients but are high in calories are said to have "empty calories." Drinks with added sugar are a typical example of empty calories. Sugar-sweetened beverages are the largest source of added sugar in the diet. In the US, the consumption of sugar-sweetened beverages has decreased modestly since around 2009; however, the intake level is still high (**Figure 9**) [95]. The Dietary Guidelines for Americans [40] and WHO [96] recommend no more than 10% of daily calories from all added sugars. As shown in **Figure 9**, adults in the US consumed an average of 160 kcal/day from sugar-sweetened beverages in 2014. This means that sugar-sweetened beverages alone correspond to 8% of total calories. To date, a large body of evidence supports a strong link between intake of sugar-sweetened beverages and weight gain [97], risk of type 2 diabetes [98], and CV diseases [99–101]. Sugar-sweetened beverages, as well as saturated fat, present a clear target for health policy.

	Complex	**Simple (or refined)**
Structure	Polysaccharides	Mono, disaccharides
Taste	No taste	Sweet
Digestion	Slow	Easy
Blood sugar	Slowly increases	Rapidly increases
Insulin response	Low	High
Glycemic index	Low	High
Satiety	Longer	Shorter
Fiber	High	Low
Body weight	Loss	Gain

Table 2.
Differences in two types of carbohydrate: complex vs. simple (refined).

Figure 8.
Changes in the format of the nutrition label of the original and the new version on Jan 2020.

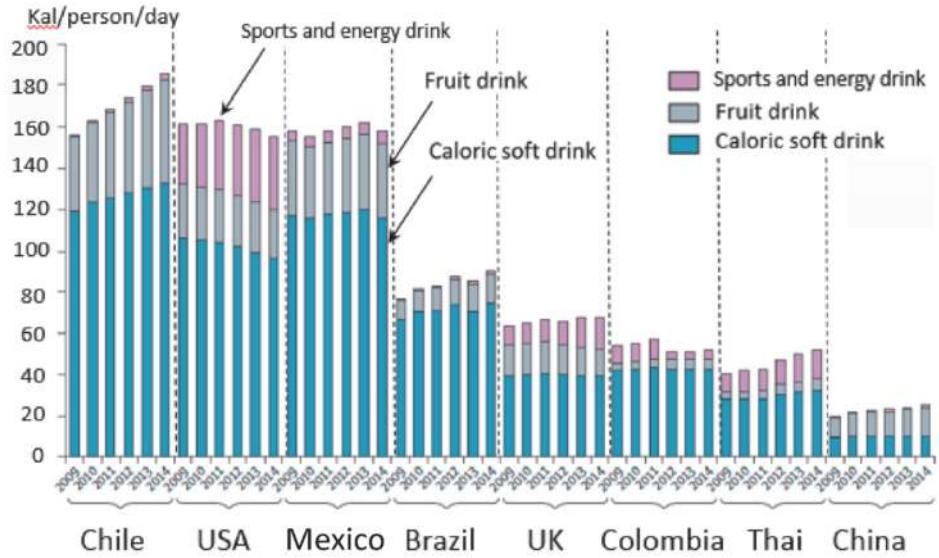

Figure 9.
Sales of sugar-sweetened beverages (SSBs) in kcal/person/day by beverage type in 2009–2014 in some selected countries [95].

3.8 Effects of replacement of SFA with other nutrients on plasma lipids, blood pressure, and other risk markers

Replacing 1% of energy from SFA with 1% of energy from PUFA, MUFA, or complex carbohydrates decreased plasma LDL cholesterol by 2.1, 1.6, and 1.3 mg/dl, respectively [102]. HDL cholesterol decreased by 0.2, 0.2, and 0.4 mg/dl after substitution of SFA by PUFA, MUFA, or complex carbohydrate. The change in TG

was −0.9, −0.4, and +1 mg/dl by substituting with PUFA, MUFA, or complex carbohydrates. These data indicate that in order to decrease LDL cholesterol or TG, the best strategy is to reduce the intake of SFA. SFA exert different LDL increasing ability depending on chain length. However, this difference is not clinically so important, because every oil contains several SFAs with various chain lengths. In general, there are progressive increases in LDL-C with diminishing chain length. The potency of the LDL-raising effects of individual SFAs is lauric acid (C12:0) > myristic acid (C14:0) > palmitic acid (C16:0) [103].

SFA may affect the risk of CV disease independently of LDL cholesterol, through the effects on inflammation, endothelial function, thrombosis, and ventricular arrhythmias [104]. One ex vivo randomized cross-over study showed that a breakfast with butter (35% SFA) induced a higher increase in TNF-alfa mRNA than breakfasts with olive oil (36% MUFA) or walnuts (16% PUFA), and also a higher response in IL-6 mRNA than the walnut breakfast in peripheral blood mononuclear cells (PBMCs) in healthy subjects (**Figure 10**) [105]. In another study, LDL-induced adhesion of monocyte to endothelial cells was also lower after MUFA consumption than after SFA in healthy individuals [106]. Some other ex vivo studies in humans have shown that consumption of butter was associated with more activated genes involved in the regulation of cell proliferation and inflammation compared with consumption of olive oil or vegetable oil [107, 108]. In these studies, activation of inflammatory markers has occurred without significant changes in the blood lipid profile [106, 108]. In summary, the previous findings strongly suggest that saturated and unsaturated fats have unique biological effects on vascular walls which are independent on the reduction in LDL cholesterol, which is like the effects of statins.

3.9 Guideline recommendations for dietary intake of SFA

Guidelines of authoritative opinions in Japan, the US, and Europe including cardiology, diabetes, and atherosclerosis societies except ADA [36] recommend less than 7% of calorie from SFA in subjects with background diseases (**Table 1**). In healthy subjects, the recommended upper limit of SFA is 7% in Japan [33] and 10% in the US [109]. Restriction of SFA to <10% rather than 7% in diabetic subjects as recommended by the ADA [36] may sound a little strange, because diabetes is associated with high CV risk, and on-target LDL cholesterol criteria is generally more stringent. This recommendation is based on the data from one small 3-week study that compared a low SFA diet (8% of total calories) vs. a high SFA diet (17% of total calories), and showed no significant difference in glycemic control and most CVD risk measures [110]. Therefore, there is limited research regarding effects of

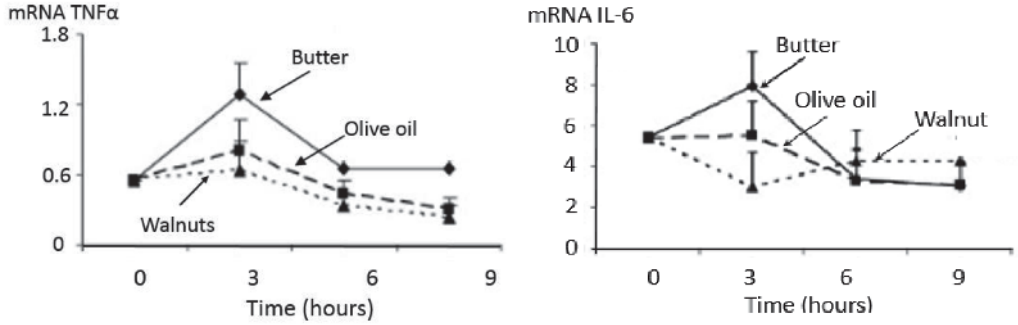

Figure 10.
Response of mRNA TNFα (left) and IL-6 (right) in breakfast with butter, olive oil, and walnuts measured in peripheral blood mononuclear cells (PBMCs) [105].

SFA on diabetic control, and the ADA nutrition position paper recommends people with diabetes follow the guidelines for the general population, which is 10% according to USDA [109]. Despite insufficient data about effects of SFA on glycemic control, the evidence in many review manuscripts or meta-analyses so far accumulated has indicated that a Mediterranean diet helps prevent type 2 diabetes and causes HbA1c reduction in persons with established diabetes [111–115]. Therefore, food habits to restrict intake of SFA as in the Mediterranean diet is a very reasonable strategy in diabetic subjects.

Three Japanese cohort studies have shown that the incidence of brain hemorrhage is inversely related to intake of SFA [116–118], and this moved the JAS to define the lower limit (4.5%) of intake of SFA [35]. This relation has not been demonstrated in meta-analysis of subjects in the US and Europe. There have been no data showing high incidence of stroke in vegetarians who consume very little SFA. Therefore, none of the guidelines in the rest of the world have incorporated the lower limit of intake of SFA. One has to be cautious that limiting SFA in the diet tends to decrease intake of good fat, as PUFA, fish oil, or olive oil.

Intake of 7% of calories as SFA is equivalent to about 15 g of SFA in a person who needs 2000 cal/day. Average intake of SFA is 15.2 and 13.8 g/day for Japanese men and women at the age of 30–49 years old [119], and 31.4 and 20.3 g/day for American men and women at the age of 31–50 years old [120]. It is reasonable that refraining from red meat, processed meat, butter, or SFA-loaded snacks is a simple way to cut SFA in daily food habits. In the US, only approximately 5% of the population consumes less than 7% of their calories from saturated fat [120].

3.10 Guideline recommendations for dietary intake of PUFA and MUFA

Beneficial effects of PUFA and MUFA seem to be fully recognized in guidelines in the US and Europe (**Table 1**). Guidelines from ADA, ACC/AHA, NLA, and USDA strongly recommend the Mediterranean and DASH diets. Regarding efficacy of decreasing blood glucose, the ADA provides the same recommendation for both PUFA and MUFA [36]. The 2013 AHA/ACC Guideline on Lifestyle Management [38] states that PUFA or MUFA should be used for reducing LDL cholesterol instead of SFA at the same recommendation level.

Literature has emerged that the biological effects of olive oil differ from those of plant-based MUFA or PUFA. Incorporating data from the PREDIMED study [6], the 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia [121] concretely recommend olive oil (>60 ml/day) and nuts (>30 g/day) rather than using general terms for MUFA. An authoritative review of dietaries and policies for cardiovascular diseases in 2016 [122] has also listed its daily recommended doses of MUFA and PUFA, such as 10–30 ml/day of soybean oil (MUFA: 22%, PUFA: 56%, and SFA: 15%), extra virgin olive oil (MUFA: 74%, PUFA: 7%, and SFA: 13%), and canola oil (MUFA: 64%, PUFA: 28%, and SFA: 7%). These guidelines provide data of effects on plasma LDL cholesterol and CV events by substitution of SFA with PUFA and MUFA.

The differences in guideline recommendations of the fat intake appear to reflect the differences in food habits and in recognition of the benefits of healthy fats between countries. In 2010, nonoptimal intake of n-6 PUFA, SFA, and trans fat resulted in 710,000, 251,000, and 537,000 deaths from CHD per year worldwide [123]. In 80% of nations, including Japan, China, and other many Asian nations, CHD burdens attributable to n-6 PUFA were 2-fold higher than the SFA-attributable burdens. These data indicate that it is more important to focus on increasing healthful n-6 rich vegetable oils in the diet than to focus on replacement of SFA and carbohydrates for public health benefits.

4. Cholesterol

4.1 Relation of plasma LDL cholesterol and dietary intake of cholesterol

Plasma LDL cholesterol value changes depending on oral intake of cholesterol. One meta-analysis of 17 studies reported that a 100 mg increment in dietary cholesterol from eggs elevated plasma total cholesterol by 2.2 mg/dl [124]. On the contrary, many studies have brought into question the apparent association between dietary cholesterol consumption and blood cholesterol [38]. Plasma cholesterol undergoes a highly degree of regulation to balance absorption in the intestine and synthesis in the liver [125]. Low dietary cholesterol intake is compensated for by an increase in absorption. These mechanisms explain the inter-individual variability in absorption (20–80%) in humans. Thus, there are responders and nonresponders to intake of cholesterol in terms of plasma cholesterol levels. Interestingly, repeated cholesterol loading has changed some nonresponders to responders [126, 127]. The presence of two types of cholesterol response may reflect just day-to-day variation of cholesterol absorption from the gut in a single person rather than a true difference between responders and nonresponders. In responders, both LDL and HDL cholesterol increased with no change in ratio of LDL to HDL. A gene of ABCG5/8 regulates absorption of dietary cholesterol and sterol from gut [128]. People carrying polymorphisms of ABCG5/8 have a higher absorption rate of cholesterol. In these subjects, the effects of manipulation of intake of cholesterol in food on plasma LDL cholesterol levels have been very significant.

The possible link between dietary cholesterol and CHD risk is potentially distorted by confounding factors from other features in the diet, especially SFA [129]. A previous recommendation of less than 300 mg of dietary cholesterol has been removed from the 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk [38], stating that there is insufficient evidence to determine whether lowering dietary cholesterol reduces LDL-C. The same action was taken in the 2015 Dietary Guidelines of USDA [40] and the 2015 Japanese dietary intake standards by Ministry of Health, Labor and Welfare [33]. By contrast, other guidelines still restrict the dietary cholesterol intake, for instance, 200 mg/day in JAS [35] and AACE [130] guidelines, or 300 mg/day in ESC/EAS [39], ADA [36], and JDS [34] guidelines. These guidelines engender the risk of potentially increasing intake of SFA by abandoning the recommendation for dietary cholesterol intake. However, all of these, in common, stress risk evaluation of atherothrombotic diseases and execution of comprehensive management to reduce the risk. Even the guideline from JAS, which limits dietary cholesterol intake at 200 mg/day, states very clearly that only restricting dietary cholesterol intake is hardly efficacious in reducing plasma LDL cholesterol, and rather more attention should be paid to reducing SFA in food.

5. Polyunsaturated fat

5.1 n-3 and n-6 PUFA

Every cooking oils or foods generally include several kinds of fatty acids. **Table 3** shows the amounts of different fatty acids in several commercially available oils and foods. In the US, people use soybean oil very frequently for cooking, and its dominant fatty acid is n-6 PUFA (50%), along with MUFA (25%). Olive oil, a very popular oil in the Mediterranean Sea area, is a well-known representative with

	Fat	SFA	MUFA	PUFA n-3	PUFA n-6
Rapeseed oil 100 g	100 g	7.4 g	63 g	9 g	18 g
Soybean oil 100 g	100 g	16 g	23 g	6.8 g	50 g
Olive oil 100 g	100 g	14 g	73 g	0.8	9.8 g
Mackerel 100 g	17 g	4.6 g (27%)	5 g (8.5%)	2.1 g (12%)	0.4 g (2.4%)
Butter 100 g	81 g	51 g (63%)	21 g (26%)	0.3 g (0.3%)	2.2 g (2.7%)
Peanuts 100 g	56 g	11 g (20%)	26 g (46%)	0 g (0%)	17 g (30%)

Figures in parenthesis (%) are the percent of calories from each fatty acid compared to the total amount of fat.

Table 3.
Amounts of different fatty acids contained in several cooking oils, butter and foods.

MUFA making up more than 70% of all fatty acids. Both soybean oil and olive oil include SFA at about 15% of all fat. It should be noted that mackerel, which people believe is a very rich source of fish oil (=n-3 PUFA), contains more than twice as much SFA and MUFA compared to n-3 PUFA. Bluefin tuna (nonoily tuna) and salmon contain only one-tenth and one-third the amount of n-3 PUFA compared with that in mackerel, respectively.

From this table, it is easily understandable that saturated fat is not synonymous with fat from animal food and likewise unsaturated fat is not synonymous with plant food. When compared gram for gram, olive oil has 7 times SFA of the trimmed beef sirloin. Furthermore, the oily fish, mackerel, which are advised to eat, has more than two times SFA of the beef sirloin. Therefore, it is not unexpected that results in clinical studies with pharmaceutical drugs with highly purified EPA differ from those of epidemiological studies with variable fish consumption. When reading manuscripts of nutritional studies with interventions involving fatty acid(s), it is important to consider which oils or food stuffs are added to modify the specific fatty acids.

The ratio of n-6 to n-3 fatty acids in the diet of early humans was estimated to be 1:1 [131]. The ratio in the diet of the US today has risen to 10:1 because of the combination of reduced n-3 fatty acid intake and the widespread use of vegetable oils rich in linoleic acid (n-6). As shown in **Table 3**, all listed foods and oils have much higher content of n-6 compared with that of n-3 except mackerel. It has been proposed that while n-3 fatty acids have anti-inflammatory effects, n-6 fatty acids have pro-inflammatory effects. This is based on data that oxylipins synthesized from n-6 PUFA have more inflammatory, vasoconstrictive, and proliferative effects compared with a metabolite derived from n-3 PUFA, although there are notable exceptions [132]. Because enzymes generating inflammatory metabolites from n-6 PUFA are inhibited by EPA and DHA from n-3 PUFA, increases in tissue concentration of EPA and DHA tend to shift the activity toward anti-inflammatory status. This is the rationale for using the ratio of n-6 to n-3 PUFA (n-6/n-3) in the blood as a potential risk marker for CV diseases, cancer, and some other chronic inflammatory diseases such rheumatoid arthritis, and bronchial asthma [133]. However, it is difficult to predict the appropriate cut-off value of n-6/n-3. Furthermore, blood concentration of n-6/n-3 does not reflect the amount of oral intake of n-3 and n-6 PUFAs [134–136]. At present, a general consensus is that high linoleic acid (n-6 PUFA) in the diet or circulation is not associated with higher in vivo or ex vivo pro-inflammatory responses, and that those individuals consuming the highest level of α-linolenic acid (n-3 PUFA) had the lowest inflammatory status [136]. Therefore, the oral intake of linoleic acid should not be restricted and it is unnecessary to consume more n-3 PUFAs.

Instead of ratio of n-6 to n-3, ω-3 index, based on measurements of EPA and DHA in red blood cells, was proposed as a marker for previous consumption of fish for last 120 days [137, 138]. The ω-3 index >8% has been proposed as optimal for cardioprotection. The average ω-3 index in Japanese is 9–11%. By contrast, the average in the US people is 4–5% and only <10% of individuals have the ω-3 index >8% [139], which reflects well the difference of fish consumption between Japan and the US.

5.2 Epidemiology and history regarding health benefits of fish oil (n-3 PUFAs)

As shown in **Table 1**, the guidelines from AHA/ACC, ADA, and AACE recommend 2 servings of fatty fish per week for the general population. A typical serving of fish can range from 3 to 6 ounces (about 85–170 g), depending on the type of fish and its preparation. Most adult Americans eat only 7–13 g/day of fish, and Japanese who are 20 years and older eat 73 g/day of fish, based on a 2013 national survey [140]. Because of the difference of daily intake of fatty food, the percent of calorie from total fat is higher among Americans than Japanese, except for n-3 PUFA, which is only 30% higher in Japanese [119, 141]. Reason for the relatively small difference in fat calorie from n-3 FUFA is that in Americans, the intake of red meat and poultry as a source of n-3 PUFA is much higher among Americans. When comparing percent of calories from EPA and DHA between Japanese and Americans, this parameter is almost four times higher in Japanese [140, 141]. The percent of calories from EPA and DHA and their concentration in the blood reflects baseline consumption of fish intake in diet.

Epidemiological data of Greenlanders from almost half century ago [142] and WHO study [143] have shown that fish consumption was independently, significantly, and inversely associated with all-cause and CHD mortality. In 1999, the GISSI-P study, the first RCT of dietary supplementation with n-3 fatty acid, showed significant reduction in the risk of the major CV events by 10% [144]. Based on the results of this RCT and positive observational studies, since 2000, the European Medicines Agency (EMA) has authorized n-3 fatty acid medicines for use after a heart attack in several EU countries, at a dose of 1 g per day. In 2002, the second AHA Science Advisory "Fish Consumption, Fish Oil, Lipids, and Coronary Heart Disease" concluded that individuals at risk for CHD benefit from the consumption of plant- and marine-derived n-3 fatty acids (1 g of EPA + DHA per day) [145].

However, since the 1990s at almost the same time as US and EU recommendations, conflicting data have been substantially reported showing no correlation between n-3 fatty acid consumption and CV events [146–154]. The Cochran meta-analysis in 2004 including 48 RCTs (36,913 participants) and 41 cohort analyses has concluded that consumption of n-3 fats did not show a reduction in the risk of total mortality or combined CV events [155]. Until the positive results in the REDUCE-IT study in 2019 [156], all major RCTs with n-3 fatty acids [157–162] except JELIS study (**Table 4**) [163], which were conducted in Japan with open-blind design. These negative results in observational studies and RCTs were enough to result in label changes in for n-3 supplements in Europe. In 2016, the ESC/EAS guidelines concluded that it was unclear whether n-3 fatty acids had beneficial effects on CV events [39]. Furthermore, 2 years later in 2018, EMA concluded that omega-3 fatty acid medicines are not effective in preventing further CV events in subjects with a history of myocardial infarction and that n-3 fatty acid supplements would no longer be authorized for secondary prevention [164]. Results of the ASCEND study [162], which was published several months before this recommendation, had substantially affected this EMA decision. The ASCEND study, which has been the largest double-blind omega-3 RCT, evaluated MACE for 7.4 years in 15,480 subjects

Study	Year	Inclusion	Statin	Follow up	Regimen	Baseline TG	Primary EP	Results
EPA/DHA study								
GISSI-P	1999	11,324 Post MI	BL = 5% EOS = 46%	3.5 y	850 mg EPA + DHA	162 mg/dl	MACE	HR = 0.85 (0.74–0.98)
GISSI-HF	2008	6975 HF	22%	3.9 y	850 mg EPA + DHA	126 mg/dl	Death + Hosp	HR = 0.91 (0.83–0.99)
OMEGA	2010	3851 Post MI	94%	1 y	840 mg EPA + DHA	121 mg/dl	Sudden death	OR = 0.95 (0.56–1.6)
Alpha-OMEGA	2010	4837 Post MI	85%	3.3 y	400 mg EPA + DHA	144 mg/dl	eMACE	HR = 1.01 (0.87–1.17)
SU.FOL.OM3	2010	2501 Post MI	85%	4.7 y	600 mg EPA + DHA	115 mg/dl	MACE	HR = 1.08 (0.79–1.47)
ORIGIN	2012	12,536 dyslipidemia + CVD	53%	6.2 y	840 mg EPA + DHA	140 mg/dl	CV death	HR = 0.98 (0.87–1.1)
Risk & Prevention	2013	12,513 High risk + CVD	41%	5 y	850 mg EPA + DHA	150 mg/dl	CV death	HR = 0.97 (0.88–1.08)
ASCEND	2018	15,480 Dm	75%	7.4 y	850 mg EPA + DHA	NA	MACE	HR = 0.9 (0.87–1.08)
Pure EPA study								
JELIS	2007	18,645 dyslipidemia	100%	4.6 y	1.8 g EPA	151 mg	eMACE	HR = 0.81 (0.69–0.95)
REDUCE-IT	2018	8179 CVD+ High risk	100%	4.9 y	4 g EPA	216 mg/dl	eMACE	HR = 0.74 (0.68–0.83)

DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, BL: baseline, EOS: end of study, EP: end point, MACE: major CV event (death, myocardial infarction, stroke), eMACE: expanded major CV event (MACE + revascularization).

Table 4.
Comparison of RCTs of n-3 PUFA [156–163].

with diabetes living in England. The n-3 supplements had no effects not only on CV events but also on mortality of other chronic diseases such as cancer and COPD. The authors also commented on the issue of cost of n-3 supplements, because up to 31% of all British people were using them. The authors clearly concluded that the recommendation of n-3 in guidelines in major meetings should be drastically corrected [162].

Compared with the clear negative opinion against effects of n-3 fatty acids from EMA, the USFDA has accepted the potential medical benefit of n-3 fatty acids for secondary prevention. In 2017, the most recent AHA Science Advisory still concluded that treatment with omega-3 PUFA supplements is reasonable for secondary prevention, stating that a potentially modest reduction in CHD mortality (10%) in this clinical population would justify treatment with a relatively safe therapy [165]. The Cochran review in 2018 (included 79 RCTs, 112,059 participants) concluded that increasing n-3 saturated fat intake made little difference to all-cause mortality and CHD mortality, which did not support the recommendation in the 2017 AHA Science Advisory of the use of n-3 fat supplements for patients with CHD [166].

Despite the inconsistent recommendation between EMA and FDA, all guidelines support an indication for reducing TG by n-3 supplements. Furthermore, they positively advise intake of fish, a rich source of marine- or plant-derived omega-3 fatty acid. The positive effects of fish consumption on CHD have been mostly attributed to n-3 PUFA (EPA and DHA); however, fish is also an excellent source of bioactive peptides which have shown beneficial activities for cholesterol-lowering, hypotensive effects through ACE inhibition, or inhibitory action for atherosclerosis, inflammation, or oxidation [167]. Supplements containing fish oil are very popular among people living in the US, being used by 7.8% of the population [168]. The reason for the popularity of n-3 supplements in the US reflects a typical lifestyle not to eat fish often (one serving of fish/week) as well as a general belief in beneficial effects of omega-3 oil for health. The commercially available n-3 supplements do not contain fish peptides but usually include at least some amount of other ingredients that are potentially harmful for health such as SFA, mercury, etc.

5.3 Reasons for inconsistent data of omega-3 fatty acids for coronary heart disease

Most of the RCTs with n-3 supplements generally have not shown a significant reduction of CV events so far. **Table 4** shows comparison of previous RCTs [144, 156–163, 169]. Inconsistent data in RCTs as well as observational studies of n-3 supplements could be attributed to statin use, dose of n-3 fatty acid, or TG and HDL values at baseline. The statin use was very low in two positive studies (GISSI-P [144] and GISSI-HF [169]), which mainly recruited subjects two decades ago, before the wide-spread use of stains. One study showed that although among statin-users coronary vascular events were not reduced with n-3 fatty acid supplements, the events significantly decreased in statin nonusers [170]. This suggests that statin treatment modified the effects of n-3 fatty acids on the incidence of major cardiovascular events.

The dosage is another important factor. Findings in two Cochran review manuscripts showed no dose dependency of n-3 fatty acids [155, 166]. It is of note that the absolute amount of fish intake was very small in some studies, which tends to make the correlation between n-3 fatty acids and CV events less clear. In some studies in cultures where eating fish is not common, the participants who belonged to the group with the highest intake of fish consumed just 2–3 servings/week, and one extreme example is a study to compare subjects who consumed no fish to subjects who ate fish once a week [148]. The JPHC study, conducted in Japan, showed clear

dose-dependency between n-3 fatty acids and CV events even in the primary prevention population [171]. Incidence of myocardial infarction was reduced by 56% in the comparison of highest (8 servings per week, or 180 g/day) vs. lowest quintiles (once a week, or 23 g/day) of fish intake. Generally speaking, a small gradient of fish consumption is associated with negative results in observational studies.

Because consumption of omega-3 fatty acids is evaluated based on self-report or food surveys by questioners, the issue of reliability about how much n-3 fatty acids was consumed has always existed. Furthermore, variability of n-3 fat content among fish species makes estimation of EPA + DHA consumption more difficult. For instance, EPA + DHA ranges from 200 to 300 mg in 3 pounds of wild tuna to more than 1500 mg in the same amount of wild mackerel.

Dose dependency of n-3 fatty acids appears to be clear in results of RCTs. As shown in **Table 4**, whereas doses of EPA/DHA used in recent negative studies were generally less than 1 g, the JELIS [163] and REDUCE-IT [156] studies, which showed significant CV risk reduction, used larger doses of EPA (see Sections 4 and 5 REDUCE-IT study). Finally, baseline TG value was suggested to affect the effects of n-3 supplements. Three positive studies, GISSI-P [144], JELIS [163], and REDUCE-IT [156] studies recruited subjects with higher baseline TG values of more than 150 mg/dl compared with the levels in other studies with negative results. In the JELIS study, whereas 1.8 g EPA reduced CV events in the whole population by 19%, the CV reduction was 53% in the subgroup with high TG (>150 mg/dl) and low HDL cholesterol (<40 mg/dl) at baseline [172] (**Figure 11**). A similar trend toward greater efficacy in subjects with higher TG and lower HDL-cholesterol at baseline was also observed in the REDUCE-IT study (**Figure 12a** and **b**) [156]. In subjects with high TG (>200 mg/dl) and low HDL cholesterol (<40 mg/dl) in the REDUCE-IT study, the percent reduction of primary endpoint by 4 g EPA was almost double (38%) compared with that in subjects with normal TG and HDL-cholesterol (21%, P for interaction = 0.04). In two fibrate studies, the BIP study (bezafibrate) [173] and the ACCORD study (fenofibrate) [174], no significant reduction of CV events reported despite the greater decrease in TG (21% in BIP and 26% in ACCORD) with higher

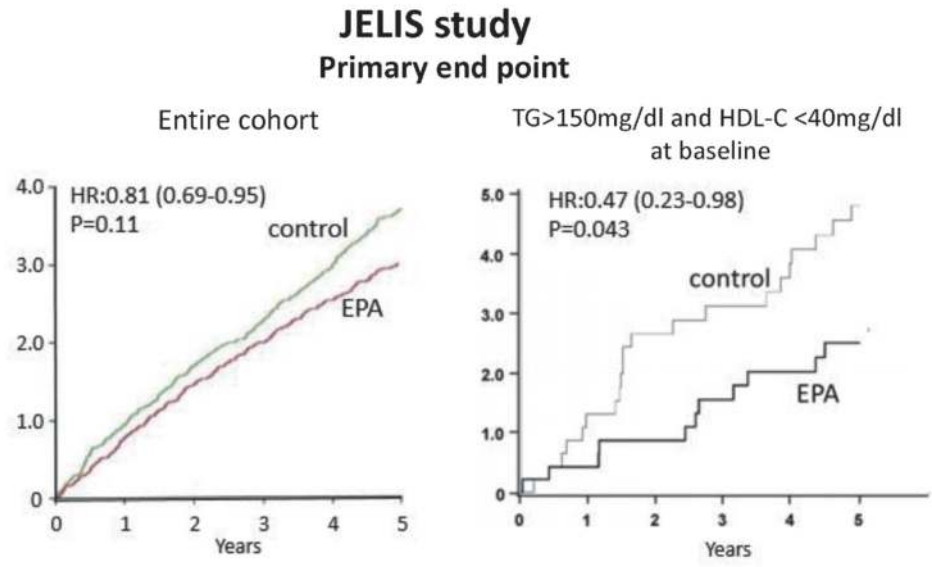

Figure 11.
Kaplan-Meier event curves for the primary end point in the entire cohort (left) and the subgroup with high TG and low HDL (right) in the JELIS study [163]. The primary end point is a composite of sudden cardiac death, nonfatal myocardial infarction, nonfatal stroke, unstable angina, and coronary revascularization.

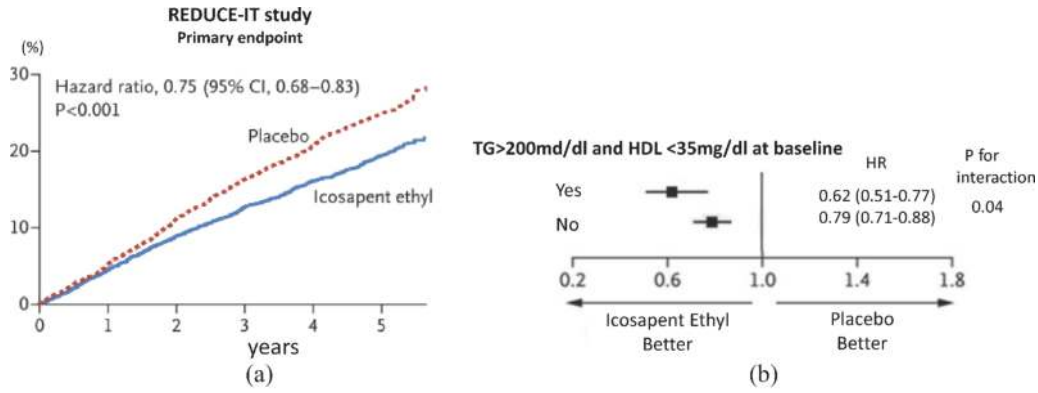

Figure 12.
(A) Kaplan-Meier event curves for the primary end point in the entire cohort in the REDUCE-IT study [156]. The end point is a composite of CV, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina. (B) The hazard ratios and 95% CI in the subgroup according to the baseline TG and HDL for the primary efficacy end point in REDUCE-IT study [156].

increases in HDL cholesterol (18% in BIP and 6% in ACCORD) compared with the REDUCE-IT study (18% decrease in TG and 3% increase in HDL). However, like the JELIS [163] and REDUCE-IT [156] studies, there was a significant reduction in CV events in subjects with higher baseline TG at >200 mg/dl [173, 174]. These data suggest that with greater abnormalities of baseline TG and HDL cholesterol, the two different dyslipidemic drugs, EPA and fibrates, tend to show greater CV events reduction. However, from the comparison of efficacy size on CV events in studies with EPA and fibrates, although at least some of the beneficial effects in the EPA studies could be explained by changing the plasma lipid, effects other than improvement of dyslipidemia are at work in the reduction of CV events by EPA.

5.4 Antithrombotic effects of n-3 fatty acids

Reduction of CV events by consumption of fish is mostly attributable to beneficial effects of n-3 fatty acids (EPA/DHA). Potential effects include antiplatelet, activity, inhibition of life-threatening arrhythmia, decrease in LDL cholesterol and TG, increase in HDL cholesterol, decrease in blood pressure, stabilization of inflammation in lipid-rich plaque, and antioxidation. Although n-3 fatty acid products are generally approved for TG lowering, this class of drugs has only modest TG lowering. Based on the modest changes in non-HDL cholesterol, reduction of CV events is calculated to be only 6–8% [175]. Therefore, changes in lipid profile alone cannot explain why EPA in the REDUCE-IT study reduced CV events by 25%.

Previous data have indicated that inhibition of platelet aggregation by n-3 fatty acids at pharmaceutical doses is insufficient for reduction of CV events. Compared with the reported value of inhibition of platelet aggregation by aspirin, prasugrel, or ticagrerol, all of which have been proven effective for reduction of CV events, antiplatelet efficacy of omega-3 fatty acids appears to be relatively small [176]. Supplementation with omega-3 fatty acids had no effect on platelet activation in the presence of aspirin [177] or clopidogrel [178]. A high level of dietary EPA + DHA (4.5, 9.5 g) or estimated biologically equivalent amounts of alpha-linolenic acid did not affect coagulation factors, fibrinogen, plasminogen activator inhibitor-1, or tissue plasminogen activator activity compared with the control [179]. All of these data suggest that antithrombotic efficacy via platelets, coagulation, and fibrinolysis does not play a major role in n-3 fatty acid-induced decrease in CV events in the positive previous studies.

5.5 REDUCE-IT study

5.5.1 Results of REDUCE-IT study

The REDUCE-IT trial has shown that use of highly purified icosapent ethyl (EPA) 2 g twice daily reduced CV events by 33% compared with placebo among patients with high TG and either known CV disease or high risk for CV disease (**Figure 12A**) [156]. The absolute reduction of the primary endpoint was 4.8%, and number needed to treat (NNT) was 21 persons/5 years (105 persons/year). This number is very excellent compared to other clinical trials recently conducted. There have been two other positive classes of drugs in addition to statins which showed significant reduction in CV events, ezetimibe and PCSK9 inhibitor. The absolute risk reduction in ezetimibe study was only 2% for 7 years (34.7–32.7%) [180]. NNT was 50 persons/7 years (350 persons/year). In the two PCSK9 inhibitors, evolocumab [181] and alirocumab [182], NNT value was 67 persons/2.2 years (147 persons/year) and 63 persons/2.8 years (176 persons/year), respectively.

5.5.2 Success factors in the REDUCE-IT study

This study had three distinctive features, compared to the previous n-3 studies that are considered to be responsible for the positive results; dose, formulation, and baseline TG. The daily dose of EPA used in the REDUCE-IT study was 4 g, more than four times higher than the doses used in previous studies. The daily dosage of n-3 supplements is usually 2 capsules containing the combination of 180 mg EPA and 120 mg DHA (=0.6 g/day of n-3), and no more than 2 g/day as dietary n-3 supplements in the FDA recommendation of 2014 [183]. The rationale of 4 g EPA in REDUCE-IT was that the blood concentration at this dose (183 μg/ml) was similar to that in Japanese subjects in JELIS study who received 1.8 g of EPA (170 μg/ml) [156, 185]. This occurred because Japanese people have much higher background intake of fish and thus have higher EPA and DHA blood concentrations than seen in Western populations. These data suggest that there may be some threshold dose to show reduction of CV events despite abundant in vitro and ex vivo data of various biological effects at smaller doses of n-3 fatty acid.

The entry criteria for TG initially was 150 mg/ml and later was changed to 200 mg/ml to increase the enrollment of patients with more significant TG elevations. Therefore, the participants' TG level at baseline was the highest (TG = 216 mg/dl) among all omega-3 studies. The ongoing STRENGTH trial, which aims to assess the efficacy and safety of a 4 g EPA/DHA combination drug, will help to clarify the influence of different formulations and dosages on CV outcomes. In January 2020, following the recommendation from DSMB, the sponsor for this study decided to close the Phase III STRENGTH trial due to its low likelihood of demonstrating a benefit to patients with mixed dyslipidemia [184]. DHA plus EPA raises LDL levels, whereas EPA alone does not raise LDL levels. The inclusion criteria for lipids were different in the two studies. REDUCE-IT had a mineral oil placebo, whereas STRENGTH had a corn oil placebo. An in vitro study has shown that EPA has direct antioxidant benefits in various apoB-containing particles that are more pronounced than those of DHA and other TG-lowering agents [185]. Although all these differences may explain partially the inconsistent results in the two studies, it appears most plausible that EPA may have different biological effects on CV events compared with those of DHA.

5.5.3 Role of EPA in management of CV disease in the future

The REDUCE-IT study is actually the first study to demonstrate significant reduction of CV events by purified EPA among Caucasians in the strong statin era.

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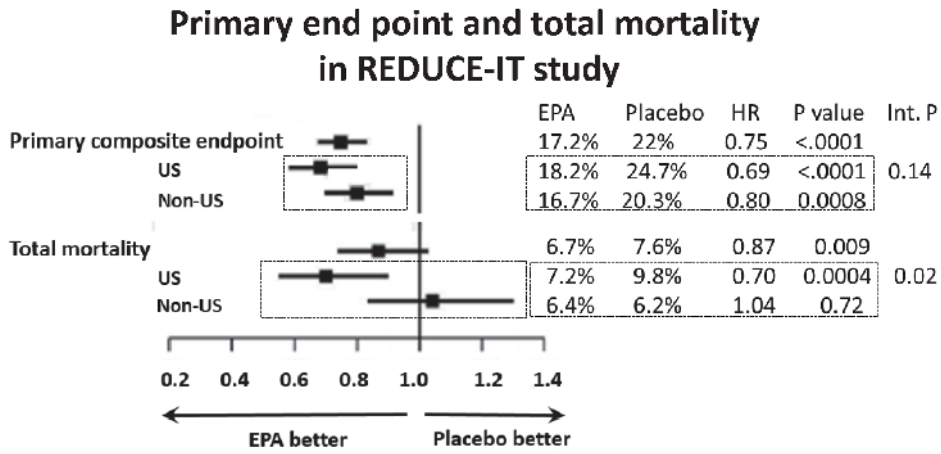

Figure 13.
The hazard ratios and 95% CI in the US and non-US population for the primary efficacy end point in REDUCE-IT study [186].

Recently, a subgroup analysis of REDUCE-IT study was conducted to determine the degree of benefit of EPA in the 3146 patients in the United States [186]. As shown in **Figure 13**, the US subgroup in REDUCE-IT demonstrated more robust risk reduction. The primary endpoint was reduced by 31% in the US cohort compared with 20% in the non-US cohort. Especially, whereas total mortality was numerically increased after 5 years in the non-US cohort, EPA reduced total mortality by 30% from 9.8 to 7.2% in the US cohort. In the current era when many clinical CV event trials have failed to show mortality benefit, this magnitude of the decrease in total mortality in the US cohort is very amazing.

Independent analysis showed that this regimen was a very rare case of high cost-effectiveness, and that EPA at $4.16/day offered better outcomes at lower health care costs for payer-eligible patients in the REDUCE IT trial [187]. Cost-performance results in clinical trials are, generally, very persuasive for obtaining regulatory approval for FDA and EMA. In December 2019, EPA became the first FDA approved drug with an indication for reduction of CV risk among patients with elevated TG levels as an add-on to maximally tolerated statin therapy [188].

A recent analysis of the REDUCE-IT study with the measurement of blood concentration of EPA has clarified the more important role of pleiotropic effects of EPA rather than lowering TG as potential mechanisms to reduce CV events [189]. The blood concentration of EPA at baseline was in a relatively narrow range and the administration of 4 g icosapent ethyl raised the blood levels by 400%. Importantly, the reduction in CV events seen in the REDUCE-IT trial was directly related to on-treatment serum levels of EPA [189]. The reduction in TG levels contributed just 2 percentage points to the overall 25 percentage points reduction in CV events in the study. This means that TG-lowering effect by EPA was not a major reason for the reduction of CV events in the REDUCE-IT study.

6. Monounsaturated fat

Compared with clinical studies on PUFA, fewer studies focusing on MUFA have been published in the literature. Whereas the replacement of SFA in the diet with MUFA increased CV events in some studies [74], other studies showed reduction [190] or no change [56, 191] in CV events. As already described, one plausible

reason for this inconsistency is the type of MUFA in the studies. It seems clear that substitution of SFA with olive oil consistently reduced CV events, meaning that it is necessary to recognize the different metabolic effects between olive oil and other oils that contain MUFA.

6.1 Lyon Diet Heart study

6.2 Results of the Lyon Diet Heart study

Despite the small decreases in LDL-cholesterol (usually <5%) with the Mediterranean diet food, in meta-analysis of many cohort studies, the magnitude of decreases in CV events was to the same extent or even larger than in statin studies [192–194]. Effects on CV events beyond LDL-cholesterol reduction with the Mediterranean diet has been demonstrated in two RCTs, the Lyon Diet Heart study and the PREDIMED study [6]. The Lyon Diet Heart study [82, 195], which was the first RCT of the Mediterranean diet before the statin era, randomized 605 patients with history of myocardial infarction to compare that diet with a French style diet. The Mediterranean diet reduced the primary endpoint by 73% (CI: 0.12–0.59, $P = 0.001$). The overall mortality was also reduced by 70% (CI: 0.11–0.82, $P = 0.02$). Although the study was originally planned to follow CV events for 5 years, it was terminated early, 2 years and 3 months after the study initiation, due to the high mortality rate in the control group. There were no significant differences in body weight, blood pressure, LDL and HDL cholesterol, and blood glucose levels between the two diet groups at the end of the study. Therefore, the marked differences in rates of CV events were not due to improvement of these classical surrogate risk markers. The investigators presented results of an extended follow-up, and the striking reduction of CV evens was maintained for up to 4 years [195].

6.2.1 Nutritional data in the intervention and control group

Table 5 shows the comparison of nutritional data in the intervention and control groups at the end of the study (27 months after the randomization) [82, 195, 196].

	Control	Intervention	*P*	To maintain desirable BW	
				Step 1	Step 2
Energy, cal	2088	1947	0.033		
Total lipids, *E*%	33.6	30.4	0.002	<30	<30
SFA, *E*%	11.7	8.0	0.0001	8–10	<7
PUFA, *E*%	6.1	4.6	0.0001	up to 10	
Linolenic (n-3), *E*%	0.29	0.84	0.0001		
Linoleic (n-6), *E*%	5.3	3.6	0.0001		
Oleic (MUFA), *E*%	10.8	12.9	0.0001	up to 15	
Carbohydrate, *E*%	49.8	53.4	N/A		
Protein, *E*%	16.6	16.2	0.3		
Fiber, g	15.5	18.6	0.004	20–30	
Cholesterol, mg	312	203	0.0001	<300	<200

Table 5.
Comparison of nutritional data in the intervention and control groups in the Lyon Diet Heart study at the end of the study (27 months after the randomization) [82, 195, 196].

In order to show the uniqueness of the intervention group in this Lyon Diet Heart study, the nutrient profiles of the NECP/AHA step 1 and step 2 are also presented. Because the intake of calories and the percentages of energy (*E*%) of three major nutrients were relatively similar, it appears that the differences in fat components had substantial effects on CV events. In the Mediterranean diet group, SFA was 32% lower (8.0 vs. 11.7%), oleic acid (MUFA) was 20% higher (12.9 vs. 10.8%), and α-linolenic acid (n-3 PUFA) was 190% higher (0.84 vs. 0.29%). Because there was no restriction of butter, fatty red meat, or snacks (rich sources of SFA) in the control diet group, and animal meat has also high content of MUFA, the energy percentage of SFA was high in the control diet group and the total unsaturated fat (MUFA + PUFA) was not different between the two groups (control vs. Mediterranean: 16.9 vs. 17.5%). The change in fat components in the Mediterranean diet group was not in accord with the current general recommendations regarding SFA and PUFA. The group had relatively high intake of SFA (8%) and lower intake of PUFA than in the control group (4.6 vs. 6.1%, **Table 4**), which could explain the small change in LDL cholesterol by the intervention. The findings in this study clearly illustrate the importance of the risk factors beyond lipids and lipoproteins.

Compared with data in PREDIMED study [6], conducted two decades later, which was loaded with extra-virgin olive oil and nuts with less refined carbohydrate, the percentage of energy of MUFA was almost half (12.9 vs. 22.1%), fat calorie was 10.8% lower (30.4 vs. 41.2%), and inversely, carbohydrate calories were 12.2% higher in the Mediterranean diet group in the Lyon Diet study (53.4 vs. 41.2%). Furthermore, the percent of calories from n-3 PUFA was low compared with those values in the REDUCE-IT [156] and JELIS [163] studies where highly purified marine-derived n-3 EPA was given. Therefore, it is unlikely that the beneficial, biological effects of n-3 PUFA or MUFA on CV risk reduction were maximally utilized for the reduction of CV events in the Lyon Diet Heart study. The very high value of SFA in the control group (11.7%) may be another factor contributing the exaggerated CV event reduction in this study. In one cohort study with 2.96 million person-years of follow-up which evaluated effects of red meat consumption on risk of CV diseases [197], the total mortality in the top quintile was 40% higher than in the bottom quintile. SFA consumption in the control group in the Lyon Diet Heart study was almost identical to that in the top quintile in that cohort study. At present, as SFA is well known to increase in LDL cholesterol, it is nearly impossible to conduct any diet trials with more than 8% intake of SFA for ethical reasons.

6.3 PREDIMED study

6.4 Protocol and results

The second RCT of the Mediterranean diet was the PREDIMED study [6]. There are several distinctions between the Lyon Diet Heart and PREDIMED studies. First, the PREDIMED study had much more participants (605 vs. 7447 subjects). Second, the Lyon Diet Heart study enrolled subjects with a history of acute myocardial infarction, and by contrast, the target is primary prevention with a high CVD risk in the PREDIMED study. Third, the Mediterranean diets were substantially different in the two studies. In the Lyon Diet Heart study, the Mediterranean diet was enriched with alpha-linolenic acid (n-3 PUFA), because margarine was provided freely in substitution for butter and cream, but not olive oil, and, by contrast, in the PREDIMED study, the diet for participants assigned to be the Mediterranean groups was loaded with extra-virgin olive oil or nuts. Finally, regarding the fat content in the control group diet, a high-fat diet was used in the Lyon Diet Heart and a low-fat diet in the PREDIMED study.

After the original version of the PREDIMED study was protocol was published in 1993 [198], statistical issues and randomization errors had been corrected, and then a revised version was published in 2018 [6]. Participants were randomized into one of three interventions: Mediterranean diet supplemented with extra-virgin olive oil (EVOO, a minimum of 50 ml/day for participants), or Mediterranean diet supplemented with nuts (30 g/day: 15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts) or control diet (advice to follow a low-fat diet). The reasons for recommending EVOO was that the content of polyphenol is quite variable among olive oils, and that EVOO has reduced CV events to a greater extent compared with non-EVOO (HR 0.84 vs. 0.97) [199]. All participants were Spanish people living in the Mediterranean area. Olive oil is a rich source of MUFA (usually more than 70% of all fat) as well as phenolic antioxidants. Walnuts contain much more PUFA (n-3: 14%, n-6: 58%) than MUFA (14%) compared with EVOO (PUFA: 13%, MUFA: 70% or more). The other half of the nut allowance was almonds and hazelnuts, both rich in MUFA (80%) and polyphenols. Thus, although having the same general food pattern as the Mediterranean diet, the EVOO group was enriched in MUFA and polyphenols and the nuts group was enriched in MUFA and (n-3, n-6) PUFA as well as polyphenols.

Figure 14 shows the daily intake of EVOO and nuts in the three groups in the PREDIMED study compared with that of Japanese aged of >60 y/o in 2015. Consumption of EVOO and nuts was very high in subjects who followed the Mediterranean diet with EVOO or nuts, respectively. It is noteworthy that even the control group had much higher consumption of EVOO than in Japanese (data on average consumption is not available; however, based on amounts of import and domestic production of olive oil in Japan, this value is very likely to be in the low single digits) and in the US population (4.2 g/day/person) [79]. Consumption of nuts was also high in the two Mediterranean groups and substantially higher in the control low-fat group than in Japanese, and probably in Americans, because according to statistical data in a US national survey in 2009–2010, 63.3% of men and 60.5% of women in the US did not consume any nuts on a given day [200]. These data indicate that all participants including those in the control cohort in this study had, to some extent, Mediterranean-style diet.

The primary endpoint (MI, stroke, and CV death) was significantly reduced, with an adjusted hazard ratio of 0.69 (CI, 0.53–0.91) for the EVOO group and 0.72 (9CI, 0.54–0.95) for the nuts group, as compared with the control diet (**Figure 2**). The Kaplan-Meier curves for the primary end point diverged soon after the trial started. The average age of participants in this study was 67 years old and a subgroup analysis showed that CV events were reduced by 30% in people more than 70 years old, similar to the value in younger people, indicating that it is never too

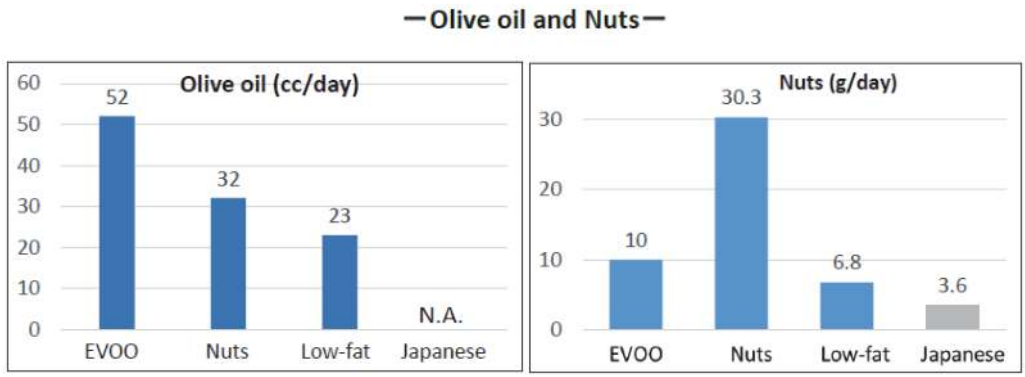

Figure 14.
Daily consumption of olive oil and nuts in the PREDIMED study at the end of the study compared with those in Japanese in their 60s [6, 140].

late to change dietary habits to improve CV health. It should be stressed that the two Mediterranean diets decreased systolic blood pressure by 5–6 mmHg, LDL- cholesterol by 5% or less, and fasting blood glucose by 2–4 md/dl, and that these small changes of risk factors alone can hardly explain the very early and significant 30% risk reduction of the primary end point.

6.4.1 Comparison to other CV studies to intervene in traditional CV risk factors

Although the primary end point was positive in the entire cohort of subjects including those in primary and secondary prevention studies, of the CANVAS program (canagliflozin) [201], the DECLARE-TIMI58 study (dapagliflozin) [202], or the LEADER study (liraglutide) [203], the subgroup analyses have shown a very small reduction in primary endpoint in subjects without established atherosclerotic diseases (**Figure 15**).

The absolute and relative risk reduction by aspirin was 0.06%/year and 12% in primary prevention group [204] and thus, because of increased bleeding complications, aspirin has not been indicated in subjects without CV diseases. What is remarkable in the PREDIMED study is that the huge reduction in CV events was

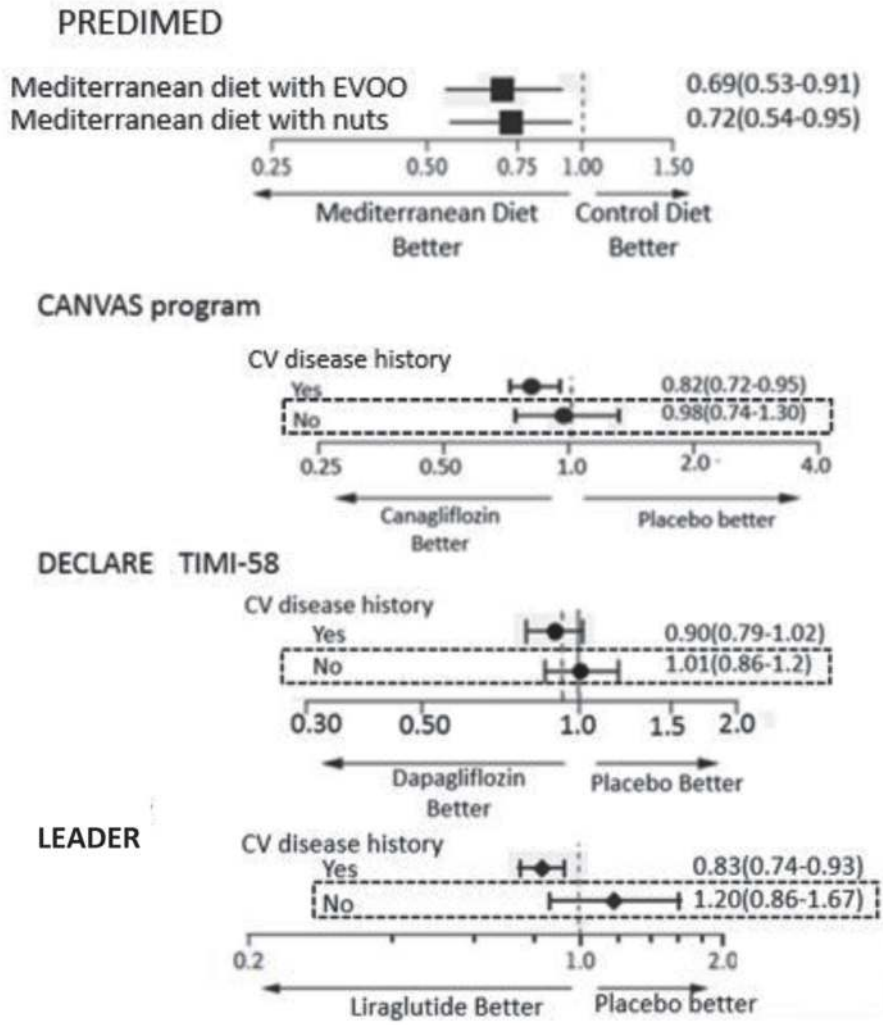

Figure 15.
The hazard ratios and 95% CI in the PREDIMED, CANVAS, DECLARE, and LEADER studies according to presence or absence of established CV diseases [6, 201–203].

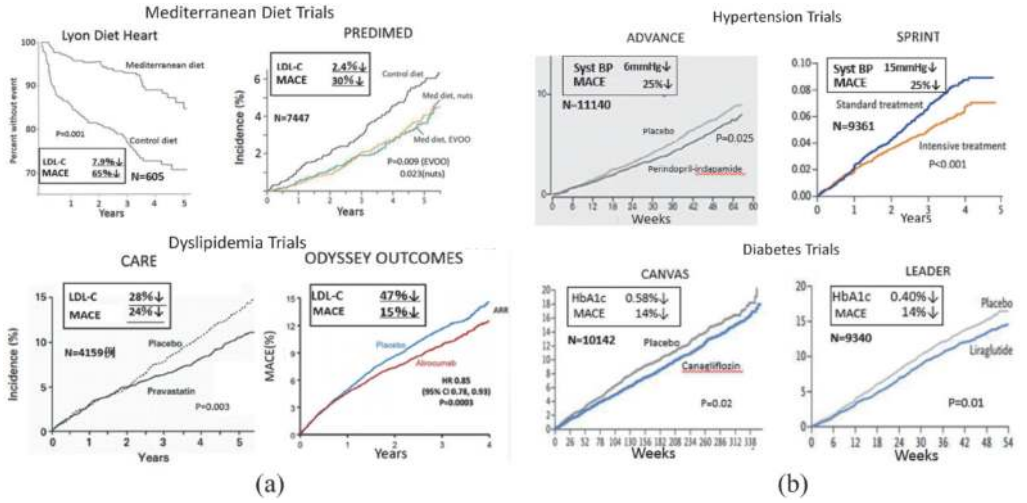

Figure 16.
(a) Kaplan-Meier event curves for the primary end point in the Lyon Diet Heart, PREDIMED, CARE, and ODYSSEY OUTCOMES studies [6, 201–203]. (b) Kaplan-Meier event curves for the primary end point in the ADVANCE, SPRINT, CANVAS, and LEADER studies [201, 203, 206, 207].

observed in the primary prevention cohort (**Figure 15**), where rates of CV events are usually low and historically, effects of drugs are rarely observed in pharmacological trials. The absolute risk reduction was almost five times higher in the PREDIMED study (0.32%/year) than that of aspirin (0.06%/year) [204].

The rapid onset of reduction in CV events should also be addressed. **Figure 16a** and **b** shows the Kaplan-Myers curves of the Lyon Diet Heart, PREDIMED, dyslipidemia (CARE [205] and ODESSEY OUTCOME [181]), hypertension (ADVANCE [206] and SPRINT [207]), and diabetes (CANVAS program [201] and LEADER [203]) studies. Whereas it takes 6 months to 2 years before the decline in CV events in most of these studies, the Kaplan-Myer curves of the two Mediterranean diet studies were separated early in the course, less than half a year. Previous studies have shown that it takes years for imaging parameters to significantly change during antihypertensive agents with carotid ultrasound [208], statin therapy [209] or an antidiabetic drug [210] with coronary intravascular ultrasound (IVUS). Considering that process in regression of plaque size needs years from studies with these image modalities, the Mediterranean diet in the PREDIMED study may be associated with early changes in plaque component, causing more stable atherosclerotic plaque which results in reduction of CV events.

6.4.2 Comparison of macronutrients between groups in PREDIMED study

Figure 17 shows comparison of total calories and percent energy from protein, fat, and carbohydrate (**Figure 17a**), and percent energy from each fatty acids (**Figure 17b**) in the PREDIMED study at the end of the study. For comparison with residents in non-Mediterranean diet cultures, data for Japanese in their 60s in 2015 [140] and US adults at age > 60 years old (total calories) and >20 years old (3 major nutrients) in 2011 [120] are also shown. In the two Mediterranean diet groups, high fat food was allowed, as long as the fat was derived from vegetable sources, particularly olive oils and nuts, and also from fatty fish. As a result, the total calories reached approximately 2200 kcal/day with fat calories exceeding more than 40% of total calories, and both of them were higher than in the low-fat control diet group (1960 kcal/day and 37%). Clearly, this was a calorie-unrestricted, high-fat diet, which is usually not recommend in obese subjects.

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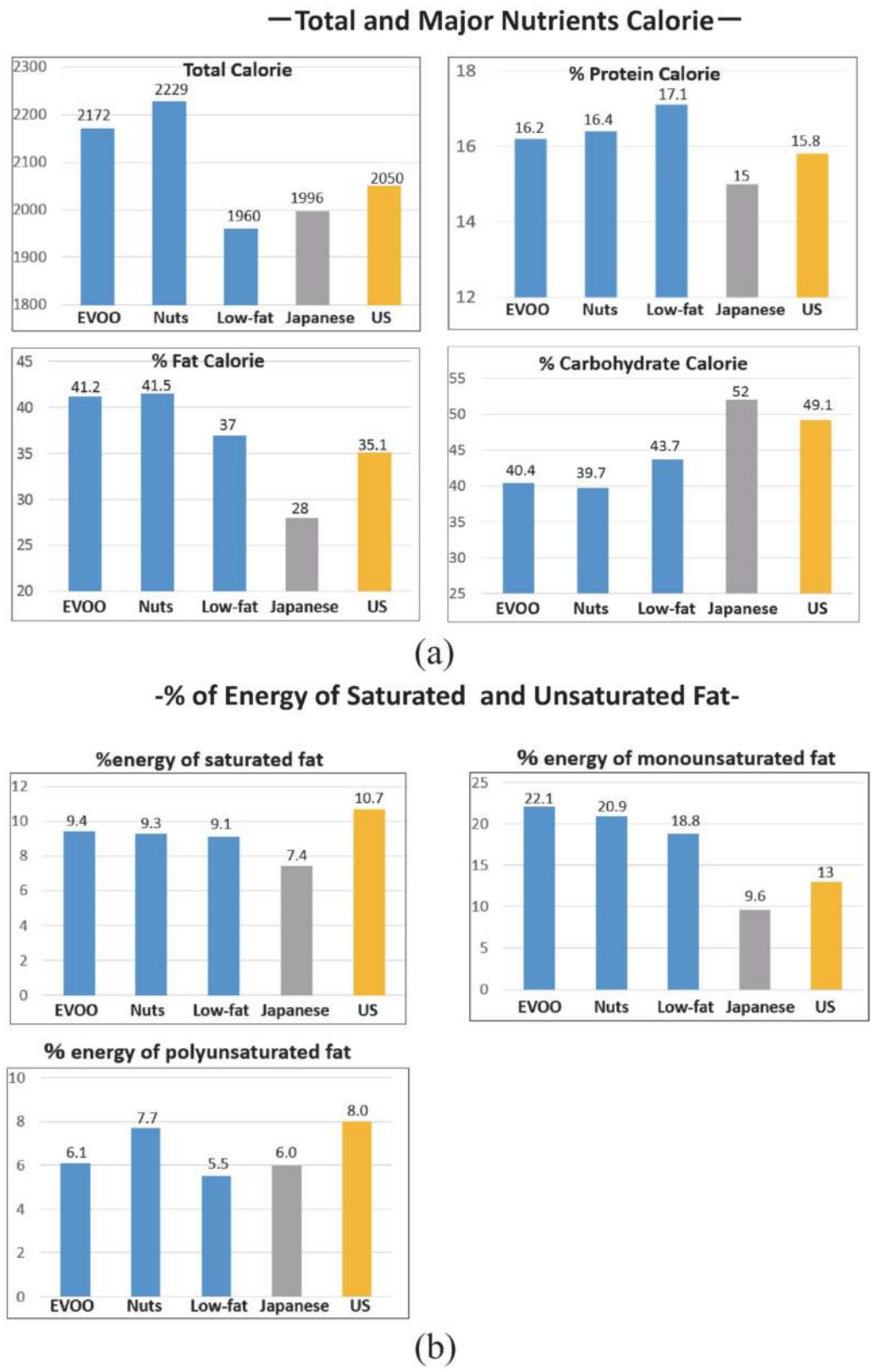

Figure 17.
(a) Total calories and percent energy from protein, fat, and carbohydrate in the PREDIMED study at the end of study [6] compared with those of Japanese in their 60s in 2015 [140] and those of US adults at age > 60 years old (total calories) and >20 years old (protein, fat, and carbohydrate) in 2011 [120]. (b) Percent energy from SFA, MUFA, and PUFA in the PREDIMED study at the end of the study compared with those of Japanese in their 60s in 2015 [140] and those of US adults at age > 20 years old in 2011 [120].

Average BMI of participants at baseline was approximately 30 and half of them fell within the class 1 obesity category (BMI > 30). A calorie-restricted diet with limited fat consumption (<30–35% of calories as fat) is usually recommended for these subjects. In the control diet group, although the focus was to reduce all types of fat and they were advised to avoid fatty foods, fat calories was elevated at 37%. This value is actually higher than in the standard low-fat diet (<30%), and higher than in Japanese and even the US adults. Therefore, all participants in the PREDIMED study represent people receiving a high fat diet. Regarding carbohydrates, in the control group, low-fat dairy products, refined carbohydrates, fruits, and vegetables were recommended as substitution for high-fat foods Therefore, a decrease in fat calories in the control group was compensated with an increase in carbohydrate calories, which was higher than the two Mediterranean diet groups. However, the level of carbohydrate calories in the control group was still lower than in Japanese and US adults.

Percent calories from fat type after 5 years of treatment in the PREDIMED study deserve attention (**Figure 17b**). The participants of all three groups were to restrict intake of red and processed meat, commercial bakery goods, sweets, and pastries, all of which are abundant sources of SFA, and were advised to select white meats (poultry without skin or rabbit). However, white meat contains a high content of SFA (30–40% of all fat). Olive oil (15%) and nuts (40% in walnuts, 7% in hazelnuts and peanuts) also contain significant amounts of SFA. As a result, the SFA was equally increased to 9% in each group, which is beyond the recommended level of SFA (<7%) in authoritative opinions. The percent SFA calories in the participants in the PREDIMED study was higher than in Japanese (in their 60s) and less than in US adults (>20 y/o).

Because of increased consumption of EVOO or nuts, MUFA was substantially higher in the EVOO and nuts group (22.1, 20.9 vs. 18.8% in control group) than in the control group. Compared with intake of MUFA in Japan (9.6%) and the US (13%), the very high percent of calories from MUFA is the most distinctive feature that characterizes the changes in nutrition in the two intervention groups. The daily recommend dose of MUFA is not defined in many guidelines except the AACE 2017 guideline which defines it as less than 10% of calories. Even in the control group, the percent of calories from MUFA (18.8%) was much higher than in subjects assigned to the Mediterranean diet in the Lyon Heart Diet study (12.9%) [82, 195]. This is because, in addition to daily intake of olive oil and nuts, many foods in Mediterranean diets are rich in MUFA, and are lower in SFA. The percent energy of PUFA was similar between the three groups and was not very different from in Japan and US. Furthermore, because the consumption of important food components for Mediterranean diet, such as fish, fruits, and vegetables (**Figure 18**), was maintained at high levels during the course of the study, the compliance in diet as described in the protocol was excellent in most participants in the PREDIMED study.

6.4.3 Potential mechanisms of reduction of CV events by olive oil and nuts

The Mediterranean diet protects against CV disease via numerous mechanisms, including reduction of blood pressure, LDL cholesterol, and blood glucose, improvement of vascular endothelium, vasodilation, anticoagulation, anti-inflammation, and antioxidant activity [192, 211]. Although components of the Mediterranean diet, which consists of fish, olive oil, vegetables, fruits, whole grains, legumes/nuts, and moderate red wine consumption, have been found to reduce CV disease risk, the general consensus is that a Mediterranean diet offers benefit against CV disease in aggregate rather than considering the effects of individual

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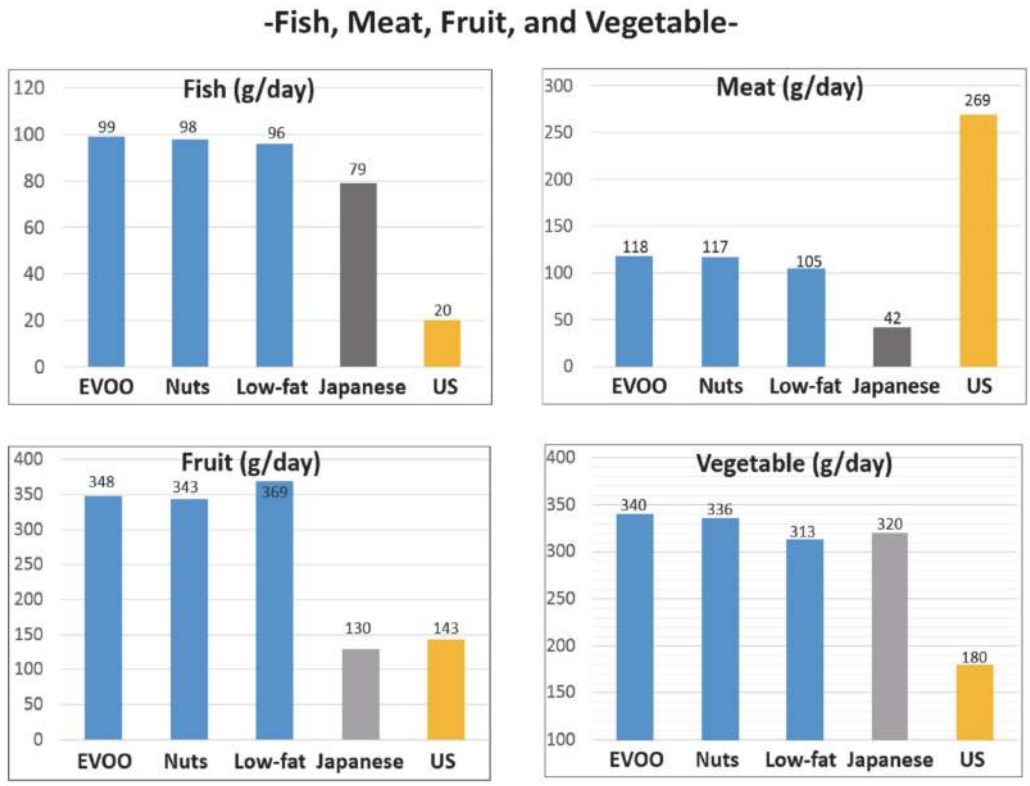

Figure 18.
Daily consumption of fish, meat, fruit, and vegetable in the PREDIMED study at the end of the study compared with those of Japanese in their 60s in 2015 [140] and those of US adults at age > 20 years old in 2011 [120].

constituents [211, 212]. This is further supported by data that the reduction of CV disease is correlated with rating for overall adherence of the Mediterranean diet [193, 213, 214]. The control group in the PREDIMED study did not consume a typical low-fat diet but rather a Mediterranean diet with mild restriction of olive oil or nuts. The subjects in the control group consumed food constituents affecting CV disease risk such as fish, vegetables, and fruits to the similar extent as in the Mediterranean diet groups except for EVOO and nuts (**Figures 14** and **18**). These findings indicate that, contrary to the previous concept of beneficial effects by synergy of all nutrients in the Mediterranean diet, extra-loaded EVOO and nuts play a critical role in the reduction of CV events in the PREDIMED study.

The key message in the PREDIMED study is that only adding EVOO or nuts to calorie-unrestricted Mediterranean diet has cut CV events by approximately 30%. High-sensitive CRP (hsCRP) in blood reflects systemic vascular inflammation. In the PROVE-IT study, 80 mg atorvastatin decreased hsCRP by 36% along with reduction of CV events by 16% [215]. In the PREDIMED study, hsCRP decreased by 50 and 40% in the EVOO and nuts groups compared with the control group [6, 198]. The magnitude of changes in hsCRP and CV events were numerically greater in the PREDIMED study than in the stain trials. These data indicate that the diet interventions have resulted in substantial improvement of vascular inflammation resulting in 30% CV event reduction. The mechanism(s) responsible for stabilization of vascular inflammation with the Mediterranean diet remains still unclear.

How about the magnitude of the well-known antioxidant effects of EVOO and nuts in the two Mediterranean diet intervention groups? The difference in amounts of polyphenol, a representative antioxidant in EVOO and nuts in the intervention groups vs. the control group, is estimated to be around 10 mg/day. Polyphenol is very abundant in other foods, such as red wine (200–400 mg/100 ml), beans (500–

1500 mg/100 g), vegetables, and fruits, and more than 1000 mg/day is usually consumed in a typical dinner on the Mediterranean diet. Furthermore, although laboratory experiments have shown beneficial effects of many antioxidants (vitamin A, C, E, NAC, polyphenol) on vascular atherosclerosis, results of human studies have generally been negative [216]. All of these data strongly suggest that the anti-oxidant effects of EVOO or nuts alone is unlikely to explain reduction in CV events observed in the two Mediterranean diet groups in the PREDIMED study.

In human, two cohort studies have shown that there was an inverse association between the dose of olive oil and CV risk [199, 217]. In the EPIC-Spain cohort study, the highest quartile of olive oil consumption (>30 g/day) was associated with a 26% reduction in risk of overall mortality and a 44% reduction in CVD mortality in comparison with non-consumers. For each increase of 10 g/day in olive oil, there was a 7% decreased risk of overall mortality and a 13% decreased risk of CVD mortality. In the PREDIMED study, the highest tertile group of mean intake of total EVOO (56.9 g/day) showed 48% reduced risk of CV mortality compared with those of the lowest tertile (21.4 g) [78]. As in previous study [21], for each 10 g/day increase in EVOO consumption, CV disease and mortality risk decreased by 10 and 7%, respectively, in the PREDIMED study. Results of all these data suggest that more CV benefit is expected for higher intake of EVOO up to 30–60 g/day.

In contrast to olive oil, the beneficial effects of nut consumption on CV disease were not consistently shown in three recent meta-analyses [218–220], whereas significant reduction in LDL cholesterol, and inflammatory and oxidants mediators were consistently reported with nuts consumption. It is important to note that the amount of walnuts consumed in previous trials was relatively large (30–108 g/day), representing 5–25% of total calories. This level of consumption appears to be difficult to maintain in a non-research setting. Recommended daily dose of nuts according to FDA is one ounce (28 g). The average nut consumption was 30 g/day in the nuts group in the PREDIMED study [6, 198]. For the first time, the PREDIMED study clearly showed that relatively small amount of nuts (30 g/day, about 180 kcal) is enough to reduce CV events.

6.4.4 Effects of olive oil in the US population and the PREDIMED-PLUS study

Although the results of the PREDIMED study have highlighted effects of olive oil and nuts, it is of note that CV benefits of olive oil and nuts were seen in conjunction with the other components of the Mediterranean diet. It still remains unclear what are the biological effects of EVOO and nuts that underlie the reduction of CV events. What is more important practically is whether similar effects on reduction in CV events is reproduced with supplements of EVOO and nuts on other types of diets, or whether lower amounts of EVOO and nuts have similar CV benefit as seen in the PREDIMED study.

All the previous studies in this area have been conducted in Mediterranean countries. The effects of olive oil on CV risk have not yet been evaluated in the U.S. population. As shown in **Figure 18**, the consumption of fish, fruit, and vegetables for the US population, important components in Mediterranean-style diet, is in the rage of 25–50% compared with that the participants in the PREDIMED study. Conversely, the consumption of meat for the US population is more than double. The critical question is whether olive oil or nuts can exhibit beneficial effects on CV risk on the background of the western style diet similarly as they have in previous observational, cohort, and RCTs of the Mediterranean diet. The recent pooled analysis from 61,181 women from the Nurses' Health Study and 31,797 men from the Health Professionals Follow-up Study has shown some answer for this

important question [79]. Replacing about 1 teaspoon per day (5 g/day) of margarine, butter, or mayonnaise, or daily fat with an equivalent amount of olive oil was associated with a 5–7% lower risk for total CV disease. When olive oil was compared with other plant-derived oils, there were no significant associations. Mean intake in the group using the highest amount of olive oil was 12 g/day in this study, not as high as in the Spanish participants of the PREDIMED study at 50 g/day. This study has provided further support for the recommendation to replace saturated fat from animal fat with plant oils, such as olive oil, that contain more MUFA for the prevention of CV disease in the general population in the US.

The protocol of the PREDIMED study did not restrict total calorie intake and did not recommend any exercise therapy. Therefore, it was criticized as showing very small beneficial effects on body weight. Although the two Mediterranean diet groups consumed 200–270 kcal more, the incidence of new diabetes was reduced about 50% compared with the control group (10.1, 11 vs. 17.9%, EVOO, nuts vs. control) [221]. If the participants in the PREDIMED study could have received calorie-restricted diet with optimal exercise therapy, then this could have resulted in fewer incidence of new patients with diabetes.

Currently another study, the PREDIMED-PLUS study, a 6-year, multicenter, randomized clinical trial for the primary prevention of CVD is ongoing [222]. The objectives of this study is to compare an energy-restricted Mediterranean diet plus advice to increase physical activities with a control, energy-unrestricted Mediterranean diet without counseling on physical activities in 6874 older individuals (www.isrctn.com/ISRCTN 89898870). The energy-reduced Mediterranean diet features more restrictive limits for red and processed meats, butter, and carbonated sweetened drinks than an unrestricted Mediterranean diet. Although this is definitely the optimal treatment for overweight or obese individuals, the major challenge is participant adherence. However, initial results from a pilot study in 626 participants showed better adherence of the energy-reduced Mediterranean group [223]. This study is expected to result in more reduction in CV events with further fine-tuning of the Mediterranean diet in the future.

7. Final remarks

The two Mediterranean diet intervention studies, the Lyon Heart Diet and PREDIMED studies, have reduced CV events by 72 and 30%, respectively. The magnitude of efficacy of these results are more powerful compared with those in statin trials. CV specialists usually lack the nutrition education to effectively implement diet therapy. In the current era of a shift from disease treatment toward prevention, how can we maintain our knowledge for updated nutritional science to provide best diet therapy to patients? Characteristics of diet therapy should be simple, understandable, and long lasting for many subjects. For this purpose, physicians must keep studying nutritional science, and should be practicing healthy diet life by themselves. A recent study using an online survey has shown that only 20% of cardiologists eat 5 servings of vegetables and fruits per day [3]. Consuming healthy food by themselves will help to more confidently and comfortably recommend appropriate diet therapy to their patients. In contrast to evidence levels in pharmacological therapy, there have been few trustworthy RCTs in nutrition, which thus has created substantial inconsistent understanding of diet therapy at present. In the future, it is by far the most important task for related parties to be united to build up a foundation of high quality data of nutritional science.

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