The Skinny on Fats: How Dietary Fats Influence Cardiovascular Disease Risk

Introduction

Poor diets are a key risk factor for cardiovascular disease (CVD), the leading cause of death among adults in developed countries. While there is no doubt that poor diets contribute to cardiovascular disease, precisely what constitutes a poor diet in terms of cardiovascular risk is still a matter of debate. Dietary fat has long been at the center of this controversy. This paper will explore the latest research regarding types and amounts of dietary fats in our diets, and how they influence our cardiovascular health.

The dietary fats at the center of the debate are saturated fatty acids, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). PUFAs are further broken down into two important groups—omega-6 and omega-3 fatty acids. Each of these types fat, depending on how much is consumed in relation to the other types, has a different effect on blood lipids (such as LDL cholesterol) as well as on other risk factors for cardiovascular disease. Therefore, the amounts and types of fat in the diet influence the risk of developing and dying from cardiovascular disease.

Yet, exactly how dietary fats effect risk and what to do about it are astonishingly difficult questions to answer. After decades of research, there are still many inconsistencies in the data. Even nutrition and health experts have not come to complete consensus around fats in the diet. Arivale's Translational Science team has examined the research to identify how different types and amounts of dietary fats influence serum lipids and risk of CVD. This paper will first summarize the evidence that connects LDL particles with CVD. Then, after briefly reviewing the detrimental impact of saturated fat, we will discuss the evidence for replacing saturated fat with other macronutrients and the resulting consequences for CVD.

Evidence that cholesterol is linked to CVD risk

There is extensive evidence that LDL cholesterol and LDL particles are a fundamental determinant of CVD risk and a causal agent in atherosclerosis—the development of plaque within arteries..[1-6] Although LDL cholesterol levels do not tell the whole story and can sometimes be misleading, evidence strongly indicates that increased LDL cholesterol is associated with an increased risk of myocardial infarction and CVD-related death.[1] Recently, genetic research has added to the already strong evidence that LDL cholesterol is a causal agent for the initial development as well as the progression of atherosclerotic plaques.[2] For example, genetic studies in individuals with defective LDL-receptor genes reveal an increased CVD mortality risk. Elevated LDL levels for prolonged periods of time further increase this risk.[3] Finally, lowering LDL cholesterol is strongly associated with reduced rates of CVD events.[4]

While LDL cholesterol concentration is a useful tool in predicting CVD risk, conventional testing does not tell the whole story. For example, LDL cholesterol concentrations can often overestimate or underestimate risk.[5] Aside from the cholesterol itself, the LDL particles, inflammation and oxidative stress, are now known to be crucial factors in arterial plaque development. Today's more advanced lipid testing can measure the number, size, and density of cholesterol-containing LDL particles.

These advanced lipid tests are more accurate at predicting CVD risk.[6] A comprehensive array of advanced lipid measurements and other biomarkers that relate to inflammation, oxidative stress and nutrient deficiencies allow for more personalized interventions.

Saturated Fat

Based on the media and popular culture's fixation with the saturated fat "controversy," one could easily assume that reducing saturated fat is no longer important for heart health. Indeed, the "butter/bacon is back" message is more ubiquitous than ever, and some believe that saturated fat, and the higher cholesterol levels that accompany it, are of no consequence in terms of CVD risk.

This recent movement fueled by both media and some health professionals is compelling at first glance. A few recently published studies conclude that there is no association between intake of saturated fat and CVD risk.[7, 8] One of these studies was a meta-analysis of observational studies reporting associations of saturated fat with all-cause mortality, CVD mortality, incidence of coronary heart disease (CHD), and stroke.[7] The researchers concluded that saturated fats are not associated with any of these outcomes. Another popular study was The Minnesota Coronary Experiment (MCE), a randomized control trial conducted in in 1968 with 9,423 men and women. Researchers recovered lost data from the MCE study, and findings were published in 2016 concluding that replacing saturated fat in the diet with PUFAs had no impact on CVD mortality, even though it did reduce cholesterol.[8] Without taking the entire body of research into account, some popular health experts cite these and other similar studies to promote the idea that reducing saturated fat does not lower CVD risk.

While these studies and arguments may seem compelling, they are not representative of the vast majority of research linking saturated fat intake with increased CVD risk. They have also been criticized for containing major flaws.[9] Critiques of the meta-analysis cited above show that the researchers base their conclusions on selected data rather than the entire set of data.[9] Some of the ignored data showed a statistically significant relationship between saturated fat intake and all-cause mortality, as well as with all of the CVD-related outcomes. The ignored data also showed that replacing dietary saturated fat with high quality carbohydrates such as fruits, vegetables, and whole grains reduced the risk of CVD. When referring to the MCE trial, many experts have cited major flaws including a significant drop in subjects (75%) due to early discharge, and fake foods created for the trial that were artificially high in omega 6's, low in omega 3's, and likely containing trans fats.[10]

While it is true that some saturated fat can certainly be a part of a healthy diet and that cholesterol is needed by the body for a number of critical biological functions, the fact remains that the vast majority of scientific evidence supports reducing saturated fat.[11-16] The largest, most detailed, and powerful study yet to examine the associations of specific dietary fats with total and cause specific mortality, just published in *JAMA Internal Medicine*, found that when compared with the same number of calories from carbohydrate, every 5% increase in saturated fat intake was associated with an 8% higher risk of overall mortality.[17] Wang et al. also found that replacing 5% of energy from saturated fats with equivalent energy from PUFA and MUFA was associated with estimated reductions in total mortality of 27% and 13%, respectively. Lastly, a meta-analysis that reviewed 15 randomized control trails (RCTs) of over 59,000 participants concluded that reducing dietary saturated fat reduced the risk of cardiovascular events by 17%.[11] Clearly, the specific nutrients that replace saturated fat are important. The next

sections of this paper will explore the research around replacing dietary saturated fats with other nutrients and the associated cardiovascular outcomes.

Replacing saturated fat with carbohydrates

Replacing saturated fat with carbohydrates can be beneficial for reducing CVD risk, but this largely depends on the type and quality of carbohydrate. As mentioned above, Wang et al. found that when compared with overall carbohydrates, every 5% increase of total calories from saturated fat was associated with an 8% higher risk of overall mortality.[17] However, intake of saturated fat, when substituted for total carbohydrates, was not significantly associated with CVD mortality. The researchers expected this lack of association with CVD because the major sources of carbohydrates in a typical Western diet are highly processed foods with large amounts of refined starch and sugar, providing a high glycemic load that can increase CVD risk independent of saturated fat.[17]

Overall, the body of research indicates that when saturated fats are replaced with refined carbohydrates, such as added sugars, CVD risk is unchanged, and some CVD risk factors, such as triglycerides and the number of small, dense LDL particles, actually increase. However, the consumption of fiber-rich, low glycemic index carbohydrates lowers LDL cholesterol, has no detrimental effects on triglycerides, and is associated with a decreased risk of heart disease.[13, 18-20]

Harvard researchers followed 84,628 women 42,908 men for 24 to 30 years and found that higher intake of carbohydrates from whole grains was significantly associated with a 10% lower risk of coronary heart disease, whereas carbohydrates from refined carbohydrates/added sugars were significantly associated with a 10% increased risk.[13] They showed that replacing 5% of energy (calorie) intake from saturated fats with equivalent energy intake from carbohydrates from whole grains was associated with a 9% lower risk of coronary heart disease.

Unfortunately, the researchers did not examine other healthy carbohydrate sources besides whole grains, like fruits, vegetables or legumes, which may very well be as beneficial as whole grains at reducing heart disease risk. However, other studies have shown that carbohydrates with a low glycemic index and/or glycemic load are associated with reduced total and LDL cholesterol as well as lower risks of myocardial infarction and coronary heart disease.[21-23] This indicates that in addition to replacing saturated fat with whole grain carbohydrates, other low glycemic carbohydrates, including many vegetables, fruits, and legumes, may also be good replacements.

Replacing saturated fat with omega-6 polyunsaturated fatty acids

The predominant unsaturated fats in the Western diet are the omega-6 polyunsaturated fatty acids (PUFA). Linoleic acid, obtained primarily from vegetable oils, nuts, and seeds, is the most abundant omega-6 PUFA. Current US dietary guidelines and the American Heart Association recommend higher intake of omega-6 PUFA to reduce the risk of coronary heart disease.[24, 25] These recommendations are based on the preponderance of evidence showing that the omega-6 PUFAs, particularly when they replace saturated fat, reduce the risk of cardiac events and deaths due coronary heart disease and improve a number of CVD risk factors, such as blood pressure and LDL cholesterol.[14, 17, 26-28] Wang et al found that among specific PUFAs, intake of linoleic acid was most strongly related to a lower risk of CVD mortality.[17] Compared to those in the lowest quintile of linoleic acid intake, those in the highest quintile

had a 22% reduced risk of CVD mortality. Similar results were found in the Cardiovascular Health Study, in which researchers followed 4707 participants for 18 years. Study participants with higher blood levels of linoleic acid had a decreased risk of death from coronary heart disease or any other cause.[27]

However, the recommendation to replace saturated fat with omega-6 PUFA remains contentious. Not all observational studies and randomized trials have shown benefits, and concerns have been raised about higher omega-6 fatty acid consumption being harmful for heart health because of potential pro-inflammatory effects.[8] Biochemically, linoleic acid can be elongated to arachidonic acid and subsequently synthesized to a variety of pro-inflammatory compounds, which could theoretically increase CVD risk. However, this speculation is not supported by randomized controlled studies, in which dietary intake of linoleic was not found to increase inflammatory markers including C-reactive protein or tumor necrosis factor-α..[29, 30] Another concern has to do with the fact that the omega-6's and omega-3's share metabolic pathways and can potentially compete with each other, causing the omega-6's to interfere with potential cardiovascular benefits of the omega-3's. However, this has not been shown to occur; rather, studies have found that the combination of both types of fatty acids is associated with the lowest levels of inflammation and the lowest risk of CVD. [27, 31]

Overall, despite the concerns, the vast majority of the research clearly points to the benefits of omega-6 fatty acids for cardiovascular health. Arivale therefore supports current dietary recommendations that emphasize replacing some saturated fat with omega-6 polyunsaturated fat. Continue on for a discussion of omega-3 PUFAs.

Omega-3 Fatty Polyunsaturated Fatty Acids

The omega-3 PUFAs, α-linoleic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are heart-healthy and should be consumed regularly for optimal cardiovascular health. EPA and DHA are primarily found in fatty fish, such as salmon, mackerel, herring, and albacore tuna, whereas ALA is primarily found in plant-based foods such as olive, canola, walnut, and flaxseed oils, as well as in walnuts, flaxseeds, and chia seeds.

The omega-3 fatty acids play an important role in preventing and treating CVD. In terms of prevention, studies show that consumption of omega-3 fatty acids in the form of fish or fish oil is associated with a decreased risk for development of coronary heart disease and death due to CVD (cardiac mortality).[32, 33] Higher levels of these fats circulating in the bloodstream are linked to a lower risk of dying from CVD or any other cause.[34] Although there are some inconsistencies, studies show that omega-3 fatty acids have beneficial effects on many CVD-related outcomes, including heart attacks and sudden cardiac death.[34-38] Omega-3 fatty acids also improve important CVD-related measurements such as fasting triglyceride levels, blood pressure, heart rate, inflammation, and blood vessel (endothelial) function.[39, 40]

Despite the many reported benefits, however, more recent trials have raised questions about the role of omega-3 fatty acids, and in particular of fish oil (EPA/DHA) supplementation, in the prevention and treatment of CVD, and there is renewed debate among experts around recommending omega-3's.[40-42] The conflicting evidence is exemplified in two recent meta-analyses which reviewed many of the same studies but come to opposite conclusions regarding the effects of EPA and DHA on CVD.[40, 43] Some of the more recent data showing limited effects may be attributed to the fact that an increasing number of study participants are on more aggressive pharmaceutical treatment, or to the fact that a number of the

studies are statistically underpowered, differ widely in EPA/DHA dosage, participants' disease states, and time of follow-up.[41, 42]

While this has undoubtedly contributed to confusion among medical professionals and the public, the current scientific literature provides strong concordant evidence that omega-3 fatty acids reduce risk of death from CVD. The strength of the data has compelled national and international guidelines to collectively recommend that healthy adults consume at least 250 mg per day of long-chain omega-3 fatty acids or at least 2 servings of oily fish per week to maintain cardiovascular health, with many organizations recommending higher amounts for those at greater risk of CVD.[44-46]

Finally, a fascinating and rapidly emerging area of research is now focused on the complex interaction between different nutrients, including omega-3 fatty acids, with relatively common mutations in genes involved in omega-3 fatty acid modulation. Results from research into these gene-nutrient interactions strongly suggests that the beneficial effects of omega-3 fatty acids are not dependent only on the intake of these essential fats, but on their interactions with genes.[47]

Replacing saturated fat with monounsaturated fat (MUFA)

Replacing saturated fats in the diet with monounsaturated fats (MUFAs), such as those found in olive oil, is associated with improvements in many cardiovascular disease-related risk factors, such as cholesterol and blood pressure, and a reduced risk of cardiovascular disease. Studies show that MUFAs are comparable to PUFAs (both linoleic and α-linolenic fatty acids) in terms of having a favorable effect on cholesterol as compared to saturated fat.[48, 49] Replacing saturated fat with MUFAs decreases total cholesterol, LDL-cholesterol, total-to-HDL cholesterol ratio, and LDL particle number.[48] Individuals who fall into the top versus bottom third of MUFA and olive oil intake have a 12% reduction in death from cardiovascular disease, a 9% reduction in cardiovascular events, such as heart attack, a 17% reduction in risk of stroke, and an 11% overall risk reduction in death from all causes.[49] Replacing just 5% of energy from saturated fat with equivalent energy from MUFAs is associated with a 13% estimated reduction in total mortality.[17]

Extra virgin olive oil, especially when consumed as part of a Mediterranean diet low in saturated fat, is associated with significantly reduced risks of cardiovascular disease and resulting deaths in individuals at high cardiovascular risk.[50] Part of what makes extra virgin olive oil such a particularly healthy source of MUFAs is the fact that it contains certain heart-healthy phytonutrients ("polyphenols") in addition to the MUFAs themselves. These polyphenols help lower total and small LDL particle numbers, lower LDL oxidation and decrease l oxidative stress, and enhance HDL function.[51-53]

Besides extra virgin olive oil, other healthy dietary sources of MUFAs include avocados and nuts such as pistachios. Replacing saturated fats with these MUFA-rich foods is a scientifically validated way to improve heart health.[54, 55]

Mediterranean Diet

The Mediterranean Diet, with its focus on fish, monounsaturated fats from olive oil, fruits, vegetables, whole grains, legumes, and nuts is arguably the best-studied and most evidence-based diet to prevent CVD. This diet is known to reduce primary cardiovascular disease outcomes such as death and events

such as stroke and myocardial infarction, as well as to improve many important biomarkers related to cardiovascular disease, including lipids and markers of inflammation.[56]

In terms of preventing cardiovascular events, results from the Prevención con Dieta Mediterránea (PREDIMED) study, published in the *New England Journal of Medicine* in 2013, showed that adhering to an energy-unrestricted Mediterranean diet supplemented with extra-virgin olive oil or mixed nuts for 4.8 years reduces the incidence of myocardial infarction, stroke and cardiovascular death by 30% compared with a control diet.[57] A follow up study that classified participants to their level of adherence to the Mediterranean diet indicated that those who had the highest adherence to a Mediterranean-type diet, had a 48% lower risk of CVD compared to those who did not follow this type of dietary pattern.[58]

Numerous meta-analysis conducted over the last decade have shown that adherence to the Mediterranean diet has reduced overall mortality and reduced CVD incidence and mortality.[59, 60] Not only does the Mediterranean diet reduce the risk of CVD by lowering LDL cholesterol concentrations, it also lowers LDL particle number[61], oxidized LDL concentrations[62], and increases LDL size.[63] In the PREDIMED study participants who supplemented their diet with nuts showed significant reductions from baseline small LDL as well as decreased LDL particle number, both of which are known to reduce CVD risk.[63]

Trans fats

Industrially produced trans fats, produced through the hydrogenation of vegetable oils, are generally found in processed baked goods and snack foods such as muffins, pies, and cakes. These can show up as "partially or fully hydrogenated vegetable oils" on ingredient labels. A 2% increase in trans-fat consumption is associated with a 23% increase in the incidence of cardiovascular disease, and a 16% increase of premature death.[17, 64] Consuming industrial trans fats has been found to increase the risk of coronary heart disease events, such as heart attacks, by 30%, and to increase the risk of death from coronary heart disease by 18%.[7] One way trans fats increase cardiovascular disease risk is by adversely affecting serum lipids, including not only traditional lipid measurements such as LDL and total cholesterol, but also more advanced lipid measurements such as LDL particle number.[65] LDL particles are directly involved in the formation of atherosclerotic plaques and, as discussed previously, LDL particle number is superior to traditional lipid markers in terms of predicting adverse events related to coronary heart disease.

Arivale recommends removing industrial trans fats from the diet completely. This recommendation is in agreement with current dietary guidelines which recommend that trans fats should be limited to less than 1% of energy or as low as possible.

Arivale's Dietary Fat Recommendations [24, 66]

Total Fat:	20-35% (Min-Max)
Saturated Fat:	<10% Total Kcal
MUFA/PUFA:	20-30% of total Kcal
Trans Fats:	Avoid whenever possible

Conclusion

Dietary fat— what types and how much—is an important consideration for cardiovascular health. While controversies surrounding dietary fat will continue as the research advances, it is currently possible to come to some evidence-based conclusions. Given the overall evidence, it is now apparent that the total amount of fat in the diet is not nearly as important as the types of fat. Lowering the amount of saturated fat consumed in the diet is beneficial for cardiovascular health. However, what replaces the saturated fat is crucial. Replacing saturated fat with PUFAs (both omega-6 and omega-3) and MUFAs reduce the risk of cardiovascular disease. Of these, the omega-3 fatty acids are the most heart-healthy, and a strong emphasis should be placed on increasing dietary omega-3 intake to optimize cardiovascular health. Replacing saturated fat with whole, unrefined carbohydrates, including whole grains, is also beneficial for reducing heart disease risk, whereas replacing saturated fat with added sugars and refined carbohydrates is deleterious and these carbohydrates should be avoided.

References

1. Collaboration PS. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths. *The Lancet.* 2007;370:1829-1839.
2. Ference BA, Yoo W, Alesh I, et al. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *Journal of the American College of Cardiology.* 2012;60:2631-2639.
3. Khera AV, Won H-H, Peloso GM, et al. Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia. *Journal of the American College of Cardiology.* 2016;67:2578-2589.
4. Trialists CT. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174 000 participants in 27 randomised trials. *The Lancet.* 2015;385:1397-1405.
5. Mora S, Buring JE, Ridker PM. Discordance of LDL cholesterol with alternative LDL-related measures and future coronary events. *Circulation.* 2013:CIRCULATIONAHA. 113.005873.
6. Cromwell WC, Otvos JD, Keyes MJ, et al. LDL particle number and risk of future cardiovascular disease in the Framingham Offspring Study—implications for LDL management. *Journal of clinical lipidology.* 2007;1:583-592.
7. de Souza RJ, Mente A, Maroleanu A, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. 2015.
8. Ramsden CE, Zamora D, Majchrzak-Hong S, et al. Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968-73). *bmj.* 2016;353:i1246.
9. Hooper L, Mann J. Observational studies are compatible with an association between saturated and trans fats and cardiovascular disease. *Evidence Based Medicine.* 2016;21:37-37.
10. Willett WC. Research Review: Old data on dietary fats in context with current recommendations. *Harvard School of Public Health.* 2016.

11. Hooper L, Martin N, Abdelhamid A, Davey Smith G. Reduction in saturated fat intake for cardiovascular disease. *The Cochrane Library.* 2015.

12. Guasch-Ferré M, Babio N, Martínez-González MA, et al. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. *The American journal of clinical nutrition.* 2015;102:1563-1573.

13. Li Y, Hruby A, Bernstein AM, et al. Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. *Journal of the American College of Cardiology.* 2015;66:1538-1548.

14. Schwab U, Lauritzen L, Tholstrup T, et al. Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type-2 diabetes, cardiovascular disease, and cancer: a systematic review. *Food & nutrition research.* 2014;58.

15. Virtanen JK, Mursu J, Tuomainen T-P, Voutilainen S. Dietary fatty acids and risk of coronary heart disease in men the Kuopio ischemic heart disease risk factor study. *Arteriosclerosis, thrombosis, and vascular biology.* 2014;34:2679-2687.

16. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Med.* 2010;7:e1000252.

17. Wang DD, Li Y, Chiuve SE, et al. Association of Specific Dietary Fats With Total and Cause-Specific Mortality. *JAMA Internal Medicine.* 2016.

18. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. *Current atherosclerosis reports.* 2010;12:384-390.

19. Siri-Tarino PW, Chiu S, Bergeron N, Krauss RM. Saturated fats versus polyunsaturated fats versus carbohydrates for cardiovascular disease prevention and treatment. *Annual review of nutrition.* 2015;35:517.

20. Te Morenga LA, Howatson AJ, Jones RM, Mann J. Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. *The American journal of clinical nutrition.* 2014;100:65-79.

21. Jakobsen MU, Dethlefsen C, Joensen AM, et al. Intake of carbohydrates compared with intake of saturated fatty acids and risk of myocardial infarction: importance of the glycemic index. *The American journal of clinical nutrition.* 2010;91:1764-1768.

22. Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *The American Journal of Clinical Nutrition.* 2000;71:1455-1461.

23. Goff L, Cowland D, Hooper L, Frost G. Low glycaemic index diets and blood lipids: a systematic review and meta-analysis of randomised controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases.* 2013;23:1-10.

24. Committee DGA. Report of the dietary guidelines advisory committee on the dietary guidelines for Americans, 2010, to the Secretary of Agriculture and the Secretary of Health and Human Services. *Agricultural Research Service.* 2010.

25. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation.* 2009;119:902-907.

26. Farvid MS, Ding M, Pan A, et al. Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies. *Circulation.* 2014:CIRCULATIONAHA. 114.010236.

27. Wu JH, Lemaitre RN, King IB, et al. Circulating omega-6 polyunsaturated fatty acids and total and cause-specific mortality: the Cardiovascular Health Study. *Circulation.* 2014:CIRCULATIONAHA. 114.011590.

28. Vafeiadou K, Weech M, Altowaijri H, et al. Replacement of saturated with unsaturated fats had no impact on vascular function but beneficial effects on lipid biomarkers, E-selectin, and blood pressure: results from the randomized, controlled Dietary Intervention and VAScular function (DIVAS) study. *The American journal of clinical nutrition.* 2015;102:40-48.

29. Johnson GH, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. *Journal of the Academy of Nutrition and Dietetics.* 2012;112:1029-1041. e1015.

30. Muka T, Kiefte-de Jong JC, Hofman A, Dehghan A, Rivadeneira F, Franco OH. Polyunsaturated Fatty Acids and Serum C-Reactive Protein The Rotterdam Study. *American journal of epidemiology.* 2015;181:846-856.

31. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation.* 2003;108:155-160.

32. König A, Bouzan C, Cohen JT, et al. A quantitative analysis of fish consumption and coronary heart disease mortality. *American journal of preventive medicine.* 2005;29:335-346.

33. Psota TL, Gebauer SK, Kris-Etherton P. Dietary omega-3 fatty acid intake and cardiovascular risk. *The American journal of cardiology.* 2006;98:3-18.

34. Mozaffarian D, Lemaitre RN, King IB, et al. Plasma phospholipid long-chain ω-3 fatty acids and total and cause-specific mortality in older adults: a cohort study. *Annals of internal medicine.* 2013;158:515-525.

35. Wen Y, Dai J, Gao Q. Effects of Omega-3 fatty acid on major cardiovascular events and mortality in patients with coronary heart disease: a meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases.* 2014;24:470-475.

36. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *The American journal of medicine.* 2002;112:298-304.

37. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n–3 fatty acids and the risk of sudden death. *New England Journal of Medicine.* 2002;346:1113-1118.

38. Siscovick DS, Raghunathan T, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *Jama.* 1995;274:1363-1367.

39. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology.* 2011;58:2047-2067.

40. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. *Jama.* 2012;308:1024-1033.

41. Sekikawa A, Doyle MF, Kuller LH. Recent findings of long-chain n-3 polyunsaturated fatty acids (LCn-3 PUFAs) on atherosclerosis and coronary heart disease (CHD) contrasting studies in Western countries to Japan. *Trends in cardiovascular medicine.* 2015;25:717-723.

42. Kromhout D, Yasuda S, Geleijnse JM, Shimokawa H. Fish oil and omega-3 fatty acids in cardiovascular disease: do they really work? *European heart journal.* 2011:ehr362.

43. Delgado-Lista J, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. *British Journal of Nutrition.* 2012;107:S201-S213.

44. Lloyd-Jones DM, Hong Y, Labarthe D, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction the American Heart Association's Strategic Impact Goal through 2020 and beyond. *Circulation.* 2010;121:586-613.

45. Authority E. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsatureated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal.* 2010;8:1461-1566.

46. Kris-Etherton PM, Harris WS, Appel LJ, Committee N. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation.* 2002;106:2747-2757.

47. Gillingham LG, Harding SV, Rideout TC, et al. Dietary oils and FADS1-FADS2 genetic variants modulate [13C] α-linolenic acid metabolism and plasma fatty acid composition. *The American journal of clinical nutrition.* 2013;97:195-207.

48. Joris PJ, Mensink RP. Role of cis-Monounsaturated Fatty Acids in the Prevention of Coronary Heart Disease. *Current Atherosclerosis Reports.* 2016;18:1-7.

49. Schwingshackl L, Hoffmann G. Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids in health and disease.* 2014;13:1.

50. Guasch-Ferré M, Hu FB, Martínez-González MA, et al. Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC medicine.* 2014;12:1.

51. Hernáez Á, Remaley AT, Farràs M, et al. Olive oil polyphenols decrease LDL concentrations and LDL atherogenicity in men in a randomized controlled trial. *The Journal of nutrition.* 2015;145:1692-1697.

52. Hernáez Á, Fernández-Castillejo S, Farràs M, et al. Olive Oil Polyphenols Enhance High-Density Lipoprotein Function in Humans A Randomized Controlled Trial. *Arteriosclerosis, thrombosis, and vascular biology.* 2014;34:2115-2119.

53. de la Torre-Carbot K, Chávez-Servín JL, Jaúregui O, et al. Elevated circulating LDL phenol levels in men who consumed virgin rather than refined olive oil are associated with less oxidation of plasma LDL. *The Journal of nutrition.* 2010;140:501-508.

54. Kasliwal RR, Bansal M, Mehrotra R, Yeptho KP, Trehan N. Effect of pistachio nut consumption on endothelial function and arterial stiffness. *Nutrition.* 2015;31:678-685.

55. Wang L, Bordi PL, Fleming JA, Hill AM, Kris-Etherton PM. Effect of a moderate fat diet with and without avocados on lipoprotein particle number, size and subclasses in overweight and obese adults: a randomized, controlled trial. *Journal of the American Heart Association.* 2015;4:e001355.

56. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. *Bmj.* 2008;337:a1344.

57. Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *New England Journal of Medicine.* 2013;368:1279-1290.

58. Martínez-González MA, Zazpe I, Razquin C, et al. Empirically-derived food patterns and the risk of total mortality and cardiovascular events in the PREDIMED study. *Clinical Nutrition.* 2015;34:859-867.

59. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *The American journal of clinical nutrition.* 2010;92:1189-1196.

60. Rees K, Hartley L, Flowers N, et al. 'Mediterranean'dietary pattern for the primary prevention of cardiovascular disease. *The Cochrane Library.* 2013.

61. Richard C, Couture P, Ooi EM, et al. Effect of Mediterranean diet with and without weight loss on apolipoprotein B100 metabolism in men with metabolic syndrome. *Arteriosclerosis, thrombosis, and vascular biology.* 2014;34:433-438.

62. Lapointe A, Goulet J, Couillard C, Lamarche Bt, Lemieux S. A nutritional intervention promoting the Mediterranean food pattern is associated with a decrease in circulating oxidized LDL particles in healthy women from the Quebec City metropolitan area. *The Journal of nutrition.* 2005;135:410-415.

63. Damasceno NR, Sala-Vila A, Cofán M, et al. Mediterranean diet supplemented with nuts reduces waist circumference and shifts lipoprotein subfractions to a less atherogenic pattern in subjects at high cardiovascular risk. *Atherosclerosis.* 2013;230:347-353.

64. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *New England Journal of Medicine.* 2006;354:1601-1613.

65. Garshick M, Mochari-Greenberger H, Mosca L. Reduction in dietary trans fat intake is associated with decreased LDL particle number in a primary prevention population. *Nutrition, Metabolism and Cardiovascular Diseases.* 2014;24:100-106.

66. Joint F. *Fats and fatty acids in human nutrition*: Karger; 2009.