

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/115445/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Ramji, Dipak P. 2019. Polyunsaturated fatty acids and atherosclerosis: insights from pre-clinical studies. *European Journal of Lipid Science and Technology* 121 (1) , 1800029. 10.1002/ejlt.201800029

Publishers page: <http://dx.doi.org/10.1002/ejlt.201800029>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Polyunsaturated fatty acids and atherosclerosis: insights from pre-clinical studies

Dipak P. Ramji*

Cardiff School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX, UK

Running title: PUFAs in atherosclerosis

Corresponding Author: Professor Dipak P. Ramji, Cardiff School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX, UK. Tel: 0044 29 20876753; Fax: 0044 29 20874116; Email: Ramji@Cardiff.ac.uk

Key words: Atherosclerosis; cardiovascular disease; nutraceuticals; polyunsaturated fatty acids; risk factors

Abbreviations: ALA, α -linolenic acid; apoE, apolipoprotein E; ARA, arachidonic acid; CARDIVEG, Cardiovascular Prevention With Vegetarian Diet; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; ECM, extracellular matrix; EPA, eicosapentaenoic acid; GISSI, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto; GLA, gamma linolenic acid; GPR, G-protein coupled receptor; IL, interleukin; JELIS, Japan EPA Lipid Intervention Study; LA, linoleic acid; LDL, low-

density lipoprotein; LDLr, LDL receptor; MAGMA, Multi-Analyte, Genetic, and Thrombogenic Markers of Atherosclerosis; MI, myocardial infarction; NF- κ B, nuclear factor-kappa B; OMEGA, Effect of Omega 3-Fatty Acids on the Reduction of Sudden Cardiac Death After Myocardial Infarction; OPTILIP, The Quantification of the Optimal n-6/n-3 ratio in the UK Diet; ORIGIN, Outcome Reduction with an Initial Glargine Intervention; oxLDL, oxidized LDL; PUFA, polyunsaturated fatty acid; REDUCE-IT, Reduction of Cardiovascular Events with EPA – Intervention Trial; STREGTH, Statin Residual Risk Reduction With EpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia; TG, triacylglycerol; Th, T-helper; Tregs regulatory T-cells; VCAM-1, vascular cell adhesion molecule 1.

Abstract

Atherosclerosis and its complications are responsible for more global deaths than any other disease. Atherosclerosis is a chronic inflammatory disease of medium and large arteries that can cause clinical complications such as myocardial infarction and cerebrovascular accidents. Current therapies against atherosclerosis mainly target the dyslipidemia associated with the disease and are associated with considerable residual risk for cardiovascular disease together with various side effects. In addition, the outcomes of clinical trials on many pharmaceutical agents against promising therapeutic targets have been disappointing. This has resulted in considerable recent interest on nutraceuticals in the prevention of atherosclerosis and as add-on with current pharmaceutical therapies. However, nutraceutical research has considerably lagged those on pharmaceuticals on two key aspects, large clinical trials and mechanistic insights. The latter forms the focus of this review in relation to the potential beneficial actions of polyunsaturated fatty acids as identified from pre-clinical studies.

Practical Applications

There is substantial recent interest in the use of nutraceuticals for the prevention and treatment of atherosclerosis. It is therefore important that the molecular mechanisms underlying their protective actions are fully understood and large clinical trials are carried out to evaluate their efficacy. Polyunsaturated fatty acids represent promising anti-atherogenic agents. This review illuminates on the mechanisms underlying their actions in relation to atherosclerosis as revealed from pre-clinical studies using *in vitro* and *in vivo* model systems.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of global morbidity and mortality [1]. According to the figures from the American Heart Association, CVD accounted for about 17.9 million deaths per year in 2015 and this is expected to increase to 23.6 million by 2030 [1]. The total direct medical costs of CVD are projected to increase to \$749 billion in 2035 thereby imposing great burden on the health care system worldwide [1].

Atherosclerosis, a chronic inflammatory disorder of medium and large arteries associated with the accumulation of lipids and fibrous elements, is the major underlying cause of CVD [2-4]. Risk factors for the disease include smoking, hypertension, hypercholesterolemia, sedentary life style, diet rich in saturated fats, diabetes, obesity and genetic predisposition [2-4]. Such risk factors, particularly the accumulation of low-density lipoproteins (LDL) in the intima of medium and large arteries that subsequently undergoes modifications such as oxidation, cause endothelial cell activation or dysfunction leading to the secretion of chemokines and the expression of adhesion proteins on the cell surface [2-4]. The net result of these changes is the recruitment of immune cells, particularly monocytes, into the subendothelial layer of the intima, which then differentiate into macrophages [2-4]. Several different macrophage phenotypes have been identified with polarization often dependent on environmental factors such as the presence of specific cytokines [5-7]. Differentiation of monocytes into macrophages is accompanied by increased expression of scavenger receptors on the cell surface that are involved in the uptake of modified LDL, particularly oxidized LDL (oxLDL) [2-4]. The uptake of LDL by their cognate LDL receptors is under negative feedback regulation whereas that by scavenger receptors is not [8]. Thus, the uncontrolled uptake of modified LDL by

scavenger receptors together with other processes (e.g. macropinocytosis) causes the transformation of macrophages into lipid laden foam cells [8]. Macrophage lipid homeostasis is maintained by control of modified LDL uptake and efflux of cholesterol from foam cells [8]. This balance is compromised during atherosclerosis resulting in excessive uptake of modified LDL [8]. Accumulation of cholesterol is toxic to the cells and triggers a range of stress responses that ultimately results in the lysis of the cells by apoptosis and necrosis [8]. This leads to the deposition of lipids that causes the formation of a lipid-rich necrotic core [8]. In addition, there is defective clearance of apoptotic body (so called efferocytosis) [2, 8]. The accumulation of lipids such as cholesterol crystals causes the activation of the inflammasome in macrophages leading to increased secretion of pro-inflammatory cytokines such as interleukin (IL)-1 and -18 [5, 7, 9]. This together with other pro-atherogenic changes results in a state of chronic inflammation that is regulated by a range of cytokines [5-9]. In particular, the cells of the adaptive immune system (e.g. T-cells, dendritic cells, B-cells etc) make a major contribution to the chronic inflammation in part via the production of such cytokines [4, 10-12]. However, the roles of different subtypes of the various classes of immune cells involved in the adaptive immune system is complex and not fully understood [4, 10-12]. For example, T-helper (Th1) cell responses are considered pro-atherogenic and regulatory T cells (Treg) anti-atherogenic whereas the roles of Th2 and Th17 cells is debatable [4, 10-12]. Similarly, B1a cells are athero-protective whereas B2 cells are pro-atherogenic [4, 11, 12]. To counteract the detrimental changes produced by the innate and the adaptive immune response, smooth muscle cells migrate from the media into the intima and produce extracellular matrix (ECM) that forms the plaque stabilizing fibrous cap [2-5]. Plaque stability is dictated by the production of ECM and its degradation by a range of proteases, particularly matrix

metalloproteinases, produced under inflammatory conditions [2-5]. Excessive degradation of ECM causes plaque rupture leading to thrombosis and subsequent clinical complications such as myocardial infarction (MI) and cerebrovascular accidents [2-5].

Although all the different stages in the pathogenesis of atherosclerosis are potential targets for therapeutic intervention, current therapies mainly regulate lipid dysfunction [2]. Statins, inhibitors of a rate limiting enzyme in the biosynthesis of cholesterol (hydroxyl methyl glutaryl CoA reductase) with additional pleiotropic actions (e.g. acting in an anti-inflammatory manner), are widely used [2]. The impact of statins in the reduction of CVD morbidity and mortality has been demonstrated by several large clinical trials [2, 13]. However, statin therapy is associated with considerable residual risk for CVD along with various side effects in some patients [13]. From the various emerging therapies, some promise has been seen with ezetimibe, which inhibits intestinal absorption of cholesterol [14], monoclonal antibodies or small interfering RNA that target the protease proprotein convertase subtilisin kexin 9 (PCSK9) [15, 16], which is involved in the degradation of the LDL receptor, and monoclonal antibodies against the pro-inflammatory cytokine IL-1 β [17]. The use of monoclonal antibodies is expensive and has to be restricted to high risk patients; this also applies with approaches that manipulate the inflammatory response as this is likely to make an individual more prone to infections [17]. Unfortunately, many other pharmaceutical leads against promising targets have failed at the clinical level, including inhibitors against cholesterol ester transfer protein and phospholipase A2 [18]. Such outcomes have therefore generated substantial interest in the use of functional foods or dietary supplements with health benefits beyond their nutritional

value (nutraceuticals) in the prevention of atherosclerosis or as add-on with current therapies such as statins [2, 3].

Diets that are rich in fruits, vegetables, fish and grains have been associated with cardiovascular health benefits [19]. For example, a recent CARDIVEG Study (Cardiovascular Prevention With Vegetarian Diet) showed that a lacto-ovo vegetarian diet was more effective at reducing LDL levels whereas a Mediterranean diet resulted in a greater reduction of triacylglycerol [20]. Several classes of nutraceuticals have been studied in relation to atherosclerosis, including polyunsaturated fatty acids (PUFAs), polyphenols, flavanols and probiotics [2, 3]. Unfortunately, research on nutraceuticals have lagged those on pharmaceuticals on two major aspects, large clinical trials and detailed mechanistic insights [2, 3]. The purpose of this review is to address our current understanding of the mechanisms underlying the actions of omega-3 and omega-6 PUFAs in atherosclerosis as identified from pre-clinical studies, particularly mouse model systems. Only brief mention will be made on the outcomes from clinical studies where the reader is directed to several recent reviews on this topic [2, 3]. Figure 1 summarizes the key atherosclerosis-associated processes where many of the PUFAs exert their beneficial actions.

2. Protective actions of omega-3 PUFAs at multiple levels

Omega-3 PUFAs are typically 18 to 22 carbon atoms in length with the first double bond beginning at the third carbon from the methyl end of the fatty acid structure (n3 position) [2, 3, 21]. The three physiologically relevant omega-3 PUFAs are α -linolenic acid (ALA; 18:3), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) [2, 3, 21]. Mammals are not able to synthesize omega-3 PUFAs but can obtain them directly from diet (dietary ALA can be metabolized to EPA and then DHA) [2, 3,

21]. Several studies have indicated anti-atherogenic actions of omega-3 PUFAs either in the form of fish oils or flax seed oils or as individual fatty acids though evidence is particularly strong for long-chain omega-3 PUFAs such as EPA and DHA [2, 3]. These studies have highlighted protective effects at multiple steps in atherogenesis [2, 3]. For example, DHA reduced tumor necrosis factor- α induced adhesion of monocytes to endothelial cells *in vitro* that was in part due to attenuation of expression of vascular cell adhesion molecule-1 (VCAM-1) and the activation of nuclear factor-kappa B (NF- κ B), a key transcription factor implicated in the control of an inflammatory response [22]. In addition, EPA and DHA attenuated the oxLDL-induced expression of adhesion molecules in human endothelial cells via the protein kinase B pathway [23]. Such an anti-atherogenic action of omega-3 fatty acids on the expression of cytokines, chemokines and adhesion proteins is not just restricted to endothelial cells but also extends to macrophages and smooth muscle cells [2, 3, 24]. Thus, omega-3 fatty acids modulate several key cellular processes associated with atherosclerosis, including attenuation of chemokine-driven monocytic migration and monocyte adhesion to endothelial cells [24, 25], inhibition of modified LDL uptake by macrophages [26], stimulation of protective, M2 macrophage polarization [27] and suppression of smooth muscle cell migration [28].

The anti-atherogenic actions of omega-3 fatty acids identified in numerous *in vitro* studies have also been seen in mouse models of atherosclerosis with effects on both the innate and adaptive immune system together with lipid metabolism and plaque stability. Two mouse model systems are commonly used for the investigation of atherosclerosis: apolipoprotein-E (apoE) deficient mice and LDL receptor (LDLr) deficient mice [2-5]. The typically slow spontaneous development of atherosclerosis in these mice can be speeded up by feeding them a western type high fat and

cholesterol containing diet [2-5]. EPA reduced atherosclerosis in both model systems and this was associated with decreased content of macrophages (less inflammation) and increased levels of smooth muscle cells and collagen indicative of plaque stability [29]. Other studies have also shown that omega-3 fatty acids ameliorate atherosclerosis in apoE^{-/-} or LDLR^{-/-} mice by reducing plasma cholesterol, monocyte recruitment to aortic lesions and favourably altering monocyte subsets by decreasing pro-atherogenic Ly6C(hi) levels [30]. In addition, EPA caused rapid regression of atherosclerosis in LDLR^{-/-} mice by modulating the phenotype of dendritic cells together with reduction in the content of macrophages and CD4(+) T cells in plaques [31]. Interestingly, a study of patients awaiting carotid endarterectomy (n=121) that were randomized to consume control capsules or omega-3 PUFA ethyl ester capsules until surgery also showed that EPA content of plaque phospholipids was inversely correlated with the number of T cells in plaque (hence adaptive immunity responses) together with plaque inflammation and instability [32]. The anti-atherogenic actions of omega-3 fatty acids are not just restricted to fish oils, DHA or EPA. For example, dietary ALA reduced atherosclerosis in apoE^{-/-} mice by decreasing T-cell driven inflammation [33]. The protective actions of omega-3 PUFAs also extend beyond atherosclerosis; for example, attenuation of aortic aneurysm development via inhibition of macrophage-mediated inflammation [34].

Several mechanisms have been proposed for the actions of omega-3 PUFAs. These include: changes in the properties of membranes of cells involved in inflammation such as their fluidity and structure of lipid rafts; effects on signal transduction pathways leading to modulation of the action of key transcription factors (e.g. inhibition of NF- κ B, activation of peroxisome proliferator-activated receptor- γ) and associated changes in the expression of downstream genes (e.g. adhesion proteins,

cytokines, chemokines); production of lipid mediators such as resolvins and maresins; and activation of G-protein coupled receptor (GPR)-120 [35]. Most of these mechanisms have been identified from *in vitro* studies so their exact importance *in vivo* remains to be fully elucidated except in a few cases. For example, resolvins D2 and E1 along with maresin 1 attenuate atherosclerosis in mouse model systems [36, 37]. Similarly, GPR120 was identified as a receptor for omega-3 PUFAs and a selective agonist for this receptor shown to improve insulin resistance and chronic inflammation in obese mice [38]. However, PUFAs-mediated activation of leukocyte GPR120 in LDLR^{-/-} mice was found to have a minimal effect on atherosclerosis [39].

The promise that omega-3 PUFAs have shown in pre-clinical studies have not been consistently translated at the clinical level [2, 3]. The discrepancies in the literature are in part due to differences in the size and composition of the participants, gender, existing risk factors for CVD, intake of pharmaceutical drugs, ethnicity, the dose of agent and the duration of the intervention [2, 3]. Promising outcomes were shown in two open-label trials, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto (GISSI)-Prevenzione trial (11,324 patients who had suffered a recent MI) and Japan EPA Lipid Intervention Study (JELIS) (18,645 hypercholesterolaemic participants) [40, 41]. Despite a smaller recruitment to JELIS, The GISSI-Prevenzione trial showed significant reduction in all-cause mortality and cardiovascular death after a relatively short period (within one year) [42] suggesting a potentially major contribution of the anti-arrhythmic effects of long-chain omega-3 PUFAs. On the other hand, the JELIS trial showed significant reduction in cardiovascular outcome after a 5-year follow-up period suggesting a major contribution of the anti-atherosclerotic properties of omega-3 PUFAs. However, both these studies were performed in a single country so differences in diet might have impacted the outcome.

Many smaller recent trials [e.g. Multi-Analyte, Genetic, and Thrombogenic Markers of Atherosclerosis (MAGMA) [43], Effect of Omega 3-Fatty Acids on the Reduction of Sudden Cardiac Death After Myocardial Infarction (OMEGA) [44] and Outcome Reduction with an Initial Glargine Intervention (ORIGIN) [45] have failed to identify any additional cardiovascular benefits of omega-3 PUFAs when taken in combination with traditional therapies. In addition, meta-analysis of 10 trials involving 77,917 individuals published this year failed to reveal any significant association between omega-3 fatty acids with fatal or non-fatal coronary heart disease (CHD) or any major vascular events [46]. Other recent meta-analysis has also shown modest reduction in cardiac death with greater benefit among higher-risk populations [47, 48]. A potential reason for the discrepancy between these recent trials and, for example, JELIS is the background dietary intake of omega-3 PUFAs (>1,000 mg/day in Japan compared to <300 mg/day in Western countries) together with the large differences in the doses of omega-3 PUFAs administered (300-900 mg/day in Western countries compared to 1,800 mg/day in the JELIS trial) [49]. Indeed, many recent, smaller randomized control trials using higher dose omega-3 PUFAs (1,800 mg/day or more) have shown significantly decreased reduction in the progression of atherosclerosis along with cardiovascular outcomes in the intervention group compared to the controls [50-53].

Increased plasma levels of triacylglycerol are an independent risk factor for CVD [4]. High-dose EPA (>3,000 mg/day) has been found to reduce the levels of triacylglycerol-rich lipoproteins without increasing LDL-cholesterol together with additional cardiovascular benefits [2, 3, 54]. However, there has previously been a lack of large, multinational, randomized clinical trials that demonstrate that lowering of triacylglycerol levels beyond that seen with statins improves the outcomes of cardiovascular events [54]. Two large clinical trials are currently investigating this key

aspect. Reduction of Cardiovascular Events with EPA – Intervention Trial (REDUCE-IT) is a randomized, double-blind, placebo-controlled trial of 8,000 participants at approximately 470 centres worldwide. The main aim of this trial is to determine whether icosapent ethyl, a highly purified ethyl ester of EPA, reduces ischemic events in patients that are currently taking statins but have high triacylglycerol levels [54]. The results from this trial are expected to be published this year. Another large international trial, STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatientS With Hypertriglyceridemia (STRENGTH), involving 13,000 patients, is investigating the effect of omega-3 carboxylic acids in combination with statins on participants with high triacylglycerol levels [55]. It is expected that this trial will be completed in 2019. Favourable outcomes in these two trials will clearly open new avenues for the use of omega-3 PUFAs in the prevention of CVD and as add-on with current therapies.

3. The good and bad aspects of omega-6 PUFAs

Omega-6 PUFAs have the final carbon-carbon double bond at the sixth bond from the methyl end (n6 position) [21]. Examples include linoleic acid (LA; 18:2), gamma linolenic acid (GLA; 18:3), dihomo-gamma-linolenic acid (DGLA; 20:3) and arachidonic acid (ARA; 20:4) [21]. In contrast to omega-3 PUFAs, omega-6 PUFAs such as ARA are precursors of potent pro-inflammatory molecules such as prostaglandins and leukotrienes [21]. Indeed, antagonistic actions have generally been attributed to omega-3 and omega-6 PUFAs in relation to the risk of CVD (anti-atherogenic and pro-atherogenic respectively) [2, 3]. For example, *in vitro* studies have shown that the omega-6 PUFA ARA increased inflammation in endothelial cells and enhanced its ability to bind to monocytes [56], potentiated cytokine-induced inflammatory processes in smooth muscle cells [57] and increased the expression of

scavenger receptors in human monocytes [58]. The omega-6 PUFA: omega 3 PUFA ratio is generally regarded as a major contributor to CVD risk with a ratio of 4:1 considered ideal for the prevention of the disease though this is debatable [59]. The excessive consumption of omega-6 PUFA-rich vegetable oils in developed countries has increased this ratio to 15:1 [60]. The importance of such a ratio in the development of atherosclerosis has been shown by several studies using mouse model systems. For example, Yamashita *et al.*, [61] varied the omega-6:omega-3 PUFA ratios in apoE⁻/LDLr⁻ double knockout mice and found that the lowest ratio was the most effective in inhibiting atherosclerotic and thrombotic parameters. Low dietary omega-6:omega-3 PUFA ratio was also associated with reduced expression of hepatic C-reactive protein, a marker of inflammation, in apoE-deficient mice [62]. In addition, reduction of dietary omega-6 PUFA: EPA plus DHA ratio in the LDLr⁻ model system decreased many markers of inflammation, macrophage cholesterol accumulation and aortic lesion formation [63]. The role of endogenous omega-6: omega-3 PUFA ratios in atherosclerosis was elegantly demonstrated by Wan *et al.*, [64] in apoE⁻ deficient mice that expressed the fat 1 gene from *Caenorhabditis elegans*. The fat-1 transgenic mice express the enzyme omega-3 fatty acid desaturase that is capable of producing omega-3 PUFAs from omega-6 PUFAs so the ratio is close to 1:1 in tissues and organs. The studies showed a decrease in atherosclerotic lesions associated with inhibition of local and systemic vascular inflammation following lowering of endogenous omega-6: omega-3 PUFA ratio. The precise mechanisms for such detrimental actions of omega-6 PUFAs are unclear though they are believed to increase the production of pro-inflammatory eicosanoids, compete with omega-3 fatty acids, which produce less inflammatory derivatives, for the same rate limiting enzymes and increase oxidation of phospholipids on lipoprotein particles [60].

Not all omega-6 PUFAs are pro-atherogenic. For example, GLA inhibited smooth muscle cell proliferation *in vitro* and attenuated the development of diet-induced atherosclerosis in apoE^{-/-} mice [65]. Similar protective actions were observed for DGLA, an elongation product of GLA [66]. Supplementation of apoE^{-/-} mice with DGLA was associated with increased acetylcholine-induced vascular inflammation and reduced plaque burden and lipid content, monocyte and macrophage numbers, migration of smooth muscle cells and the expression of intercellular adhesion protein-1 and VCAM-1 [67]. The anti-atherogenic action of DGLA was attenuated by a non-selective cyclooxygenase inhibitor naproxen [66].

The outcome on the impact of omega-6 PUFAs on CVD and related parameters from clinical studies have not been consistent. The Quantification of the Optimal n-6/n-3 ratio in the UK Diet (OPTILIP) study of 258 subjects investigated the impact of altering the ratio of omega-6 to omega-3 PUFAs [67, 68]. The study showed that decreasing the omega-6: omega 3 PUFA ratio to approximately 3:1 reduced plasma levels of triacylglycerol and produced favourable changes in LDL size without influencing hemostatic risk factors (e.g. fibrinogen, clotting factors VII and XII) and insulin sensitivity [67, 68]. Pooled data from 11 cohort studies involving 340,000 individuals demonstrated a reduction of CHD with a low intake of saturated fats and a proportionally higher intake of omega-6 PUFAs [69, 70]. Similarly, a systematic review and meta-analysis of randomized controlled trials involving 13,614 participants showed reduced CHD events by consumption of PUFAs instead of saturated fats [71]. However, a meta-analysis of randomized controlled trials published last year suggested that replacing saturated fats with mostly omega-6 PUFAs was unlikely to decrease CHD events, CHD mortality or even total mortality [72]. In addition, a systematic review and meta-analysis involving 32 observational studies (530,525

participants) on dietary intake of fatty acids, 17 observational studies (25,721 participants) of fatty acid biomarkers and 27 randomized control trials (103,052 participants) of fatty acid supplementation failed to support cardiovascular guidelines that encourage high consumption of PUFAs and low consumption of saturated fats [73]. In relation to specific PUFAs, this study did find that EPA, DHA and ARA were associated with lower risk of coronary events [73]. The Nurses' Health Study of 78,778 US women over a 20 years follow-up revealed an inverse association between the intake of linoleic acid, an omega-6 PUFA, and the risk of CHD [74]. Similarly, a systematic review and meta-analysis of prospective cohort studies involving 310,602 individuals showed that dietary linoleic acid was inversely associated with CHD risk in a concentration-dependent manner [75]. In addition, a population-based study of 1,551 middle-aged men over a 15-year follow up showed protective effects of dietary linoleic acid and CVD risk and total mortality [76]. On the other hand, a study of 2,206 healthy Japanese men found that serum omega-6 fatty acid levels produced unfavourable conditions such as CRP levels and arterial stiffness/wave reflection though this study did not directly determine plasma linoleic acid levels [77]. The discrepancies in the literature detailed above highlight the need for large, controlled clinical trials to evaluate the impact of omega-6 fatty acids on CVD.

4. Conclusions

Current therapies for atherosclerosis are associated with a substantial residual CVD risk and whilst there have been some recent successes on emerging therapies, many promising leads have failed at the clinical level. In addition, current therapies also suffer from other issues such as adverse side effects in some cases. Major recent interest has therefore been generated on the use of nutritional products, which are

known to have excellent safety profile, for the prevention of atherosclerosis and as add-on with current therapies. PUFAs represent promising nutraceuticals and indeed numerous pre-clinical studies highlighted in this review have shown beneficial actions of omega-3 PUFAs and some omega-6 PUFAs (e.g. DGLA) on multiple risk factors or cellular processes associated with atherosclerosis. However, mechanistic insight still remains relatively limited, so it is essential that this is addressed in the future particularly using doses that are in the physiological range and can be translated to humans. Indeed, the promise shown in pre-clinical research have not been consistently observed in human studies and the discrepancies probably reflects differences in the size of the cohort (mostly at the lower end), the dose and duration of the intervention, heterogeneity of the cohorts, background from current therapies and limited parameters studied. However, the outcome of the large REDUCE-IT and STRENGTH trials that are currently under way for omega-3 PUFA supplementation is likely to open several similar scale, controlled trials on other nutraceuticals such as some omega-6 PUFAs. The next few years indeed represent exciting for nutraceutical research, particularly on PUFAs.

Acknowledgements

Research in the laboratory was funded in part by grants from the British Heart Foundation (PG/16/25/32097 and FS/17/75/33257).

Conflict of interest

DPR has received funding for PhD Studentships from Cultech Limited.

References

- 1 Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., de Ferranti, S. D., Ferguston, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jiménez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., Lichtman, J. H., Lisabeth, L., Liu, S., Longenecker, C. T., Lutsey, P. L., Mackey, J. S., Matchar, D. B., Matsushita, K., Mussolino, M. E., Nasir, K., O'Flaherty, M., Palaniappan, L. P., Pandey, A., Pandey, D. K., Reeves, M. J., Ritchey, M. D., Rodriguez, C. J., Roth, G. A., Rosamond, W. D., Sampson, U. K. A., Satou, G. M., Shah, S. H., Spartano, N. L., Tirschwell, D. L., Tsao, C. W., Voeks, J. H., Willey, J. Z., Wilkins, J. T., Wu, J. H., Alger, H. M., Wong, S. S., Muntner, P; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2018 Update: A report from the American Heart Association. *Circulation*. 2018, 137, e67-e492.
- 2 Moss, J. W. E., Williams, J. O., and Ramji, D. P., Nutraceuticals as therapeutic agents for atherosclerosis. *Biochim. Biophys. Acta*. 2018, 1864, 1562-1572.
- 3 Moss, J. W. E., and Ramji, D. P., Nutraceutical therapies for atherosclerosis. *Nat. Rev. Cardiol*. 2016, 13, 513-532.
- 4 Buckley, M. L., and Ramji, D. P., The influence of dysfunctional signaling and lipid homeostasis in mediating the inflammatory responses during atherosclerosis. *Biochim. Biophys. Acta*. 2015, 1852, 1498-1510.
- 5 Ramji, D. P., and Davies, T. S., Cytokines in atherosclerosis: Key players in all stages of disease and promising therapeutic targets. *Cytokine Growth Factor Rev*. 2015, 26, 673-685.

- 6 Salter, R. C., Foka, P., Davies, T. S., Gallagher, H., Michael, D. R., Ashlin, T. G., and Ramji, D. P., The role of mitogen-activated protein kinases and sterol receptor coactivator-1 in TGF- β -regulated expression of genes implicated in macrophage cholesterol uptake. *Sci. Rep.* 2016, 6, 34368.
- 7 Moss, J. W. E, and Ramji, D. P., Cytokines: roles in atherosclerosis disease progression and potential therapeutic targets. *Future Med. Chem.* 2016, 8, 1317-1330.
- 8 McLaren, J. E., Michael, D. R., Ashlin, T. G., and Ramji, D. P., Cytokines, macrophage lipid metabolism and foam cells: Implications for cardiovascular disease therapy. *Prog. Lipid Res.* 2011, 50, 331-347.
- 9 Shapiro, M. D., and Fazio, S., From lipids to inflammation: New approaches to reducing atherosclerotic risk. *Circ. Res.* 2016, 118, 732-749.
- 10 Tabas, I., and Lichtman, A. H., Monocyte-macrophages and T cells in atherosclerosis. *Immunity* 2017, 47, 621-634.
- 11 Ketelhuth, D. F., and Hansson, G. K., Adaptive response of T and B cells in atherosclerosis. *Circ. Res.* 2016, 118, 668-678.
- 12 Sage, A. P., and Mallat, Z., Readapting the adaptive immune response - therapeutic strategies for atherosclerosis. *Br. J. Pharmacol.* 2017, 174, 3926-3939.
- 13 Ramkumar, S., Raghunath, A., and Raghunath, S., Statin therapy: Review of safety and potential side effects. *Acta Cardiol. Sin.* 2016, 32, 631-639.
- 14 Murphy, S. A., Cannon, C. P., Blazing, M. A., Giugliano, R. P., White, J. A., Likhnygina, Y., Reist, C., Im, K., Bohula, E. A., Isaza, D., Lopez-Sendon, J., Dellborg, M., Kher, U., Tershakovec, A. M., and Braunwald, E. Reduction in total cardiovascular events with ezetimibe/simvastatin post-acute coronary syndrome: The IMPROVE-IT trial. *J. Am. Coll. Cardiol.* 2016, 67, 353-361.

- 15 Sabatine, M. S., Giugliano, R. P., Keech, A. C., Honarpour, N., Wiviott, S. D., Murphy, S. A., Kuder, J. F., Wang, H., Liu, T., Wasserman, S. M., Sever, P. S., Pedersen, T. R; FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N. Engl. J. Med.* 2017, 376, 1713-1722.
- 16 Ray, K. K., Landmesser, U., Leiter, L. A., Kallend, D., Dufour, R., Karakas, M., Hall, T., Troquay, R. P., Turner, T., Visseren, F. L., Wijngaard, P., Wright, R. S., and Kastelein, J. J. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N. Engl. J. Med.* 2017, 376, 1430-1440.
- 17 Ridker, P. M., Everett, B. M., Thuren, T., MacFadyen, J. G., Chang, W. H., Ballantyne, C., Fonseca, F., Nicolau, J., Koenig, W., Anker, D. D., Kastelein, J. J. P., Cornel, J. H., Pais, P., Pella, D., Genest, J., Cifkova, R., Lorenzatti, A., Forster, T., Kobalava, Z., Vida-Simiti, L., Flather, M., Shimokawa, H., Ogawa, H., Dellborg, M., Rossi, P. R. F., Troquay, R. P. T., Libby, P., Glynn, R. J; CANTOS trial group. Antiinflammatory therapy with Canakinumab for atherosclerotic disease. *N. Engl. J. Med.* 2017, 377, 1119-1131.
- 18 Ladeiras-Lopes, R., Agewall, S., Tawakol, A., Staels, B., Stein, E., Mentz, R. J., Leite-Moreira, A., Zannad, F., and Koenig, W. Atherosclerosis: recent trials, new targets and future directions. *Int. J. Cardiol.* 2015, 192, 72-81.
- 19 Ravera, A., Carubelli, V., Sciatti, E., Bonadei, I., Gorga, E., Cani, D., Vizzardì, E., Metra, M., and Lombardi, C. Nutrition and cardiovascular disease: Finding the perfect recipe for cardiovascular health. *Nutrients.* 2016, 8, E363.
- 20 Sofi, F., Dinu, M., Pagliai, G., Cesari, F., Gori, A. M., Sereni, A., Becatti, M., Fiorillo, C., Marcucci, R., and Casini, A. Low-calorie vegetarian versus Mediterranean diets for reducing body weight and improving cardiovascular risk profile: CARDIVeG

Study (Cardiovascular Prevention With Vegetarian Diet). *Circulation*. 2018, 137, 1103-1113.

21 Tortosa-Caparrós, E., Navas-Carrillo, D., and Orenes-Piñero, E. Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Crit. Rev. Food Sci. Nutr.* 2017, 57, 3421-3429.

22 Wang, T. M., Chen, C. J., Lee, T. S., Chao, H. Y., Wu, W. H., Hsieh, S. C., Sheu, H. H., and Chiang, A. N., Docosahexaenoic acid attenuates VCAM-1 expression and NF- κ B activation in TNF- α -treated human aortic endothelial cells. *J. Nutr. Biochem.* 2011, 22, 187-194.

23 Chen, H., Li, D., Chen, J., Roberts, G. J., Saldeen, T., and Mehta, J. L., EPA and DHA attenuate ox-LDL-induced expression of adhesion molecules in human coronary artery endothelial cells via protein kinase B pathway. *J. Mol. Cell. Cardiol.* 2003; 35, 769-775.

24 Moss, J. W. E., Davies, T. S., Garaiova, I., Plummer, S. F., Michael, D. R., and Ramji, D. P., A unique combination of nutritionally active ingredients can prevent several key processes associated with atherosclerosis *in vitro*. *PLoS One*, 2016, 11, e0151057.

25 Yamada, H., Yoshida, M., Nakano, Y., Suganami, T., Satoh, N., Mita, T., Azuma, K., Itoh, M., Yamamoto, Y., Kamei, Y., Horie, M., Watada, H., and Ogawa, Y. *In vivo* and *in vitro* inhibition of monocyte adhesion to endothelial cells and endothelial adhesion molecules by eicosapentaenoic acid. *Arterioscler. Thromb. Vasc. Biol.* 2008, 28, 2173-2179.

- 26 McLaren, J. E., Michael, D. R., Guschina, I. A., Harwood, J. L., and Ramji, D. P., Eicosapentaenoic acid and docosahexaenoic acid regulate modified LDL uptake and macropinocytosis in human macrophages. *Lipids*. 2011, *46*, 1053-1061.
- 27 Chang, H. Y., Lee, H. N., Kim, W., and Surh, Y. J., Docosahexaenoic acid induces M2 macrophage polarization through peroxisome proliferator-activated receptor γ activation. *Life Sci*. 2015, *120*, 39-47.
- 28 Mizutani, M., Asano, M., Roy, S., Nakajima, T., Soma, M., Yamashita, K., and Okuda, Y., Omega-3 polyunsaturated fatty acids inhibit migration of human vascular smooth muscle cells *in vitro*. *Life Sci*. 1997, *61*, PL269-274.
- 29 Matsumoto, M., Sata, M., Fukuda, D., Tanaka, K., Soma, M., Hirata, Y., and Nagai, R., Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. *Atherosclerosis*. 2008, *197*, 524-533.
- 30 Brown, A. L., Zhu, X., Rong, S., Shewale, S., Seo, J., Boudyguina, E., Gebre, A. K., Alexander-Miller, M. A., and Parks, J. S. Omega-3 fatty acids ameliorate atherosclerosis by favorably altering monocyte subsets and limiting monocyte recruitment to aortic lesions. *Arterioscler. Thromb. Vasc. Biol*. 2012, *32*, 2122-2130.
- 31 Nakajima, K., Yamashita, T., Kita, T., Takeda, M., Sasaki, N., Kasahara, K., Shinohara M., Rikitake, Y., Ishida, T., Yokoyama, M., and Hirata K. Orally administered eicosapentaenoic acid induces rapid regression of atherosclerosis via modulating the phenotype of dendritic cells in LDL receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol*. 2011, *31*, 1963-1972.
- 32 Cawood, A. L., Ding, R., Napper, F. L., Young, R. H., Williams, J. A., Ward, M. J., Gudmundsen, O., Vige, R., Payne, S. P., Ye, S., Shearman, C. P., Gallagher, P. J., Grimble, R. F., and Calder, P. C., Eicosapentaenoic acid (EPA) from highly concentrated n-3 fatty acid ethyl esters is incorporated into advanced atherosclerotic

plaques and higher plaque EPA is associated with decreased plaque inflammation and increased stability., *Atherosclerosis* 2010, 212, 252-259.

33 Winnik, S., Lohmann, C., Richter, E. K., Schäfer, N., Song, W. L., Leiber, F., Mocharla, P., Hofmann, J., Klingenberg, R., Borén., Becher, B., Fitzgerald, G. A., Lüscher, T. F., Matter, C. M., and Beer, J. H. Dietary α -linolenic acid diminishes experimental atherogenesis and restricts T cell-driven inflammation. *Eur. Heart J.* 2011, 32, 2573-2584.

34 Yoshihara, T., Shimada, K., Fukao, K., Sai, E., Sato-Okabayashi, Y., Matsumori, R., Shiozawa, T., Alshahi, H., Miyazaki, T., Tada, N., and Daida, H. Omega 3 polyunsaturated fatty acids suppress the development of aortic aneurysms through the inhibition of macrophage-mediated inflammation. *Circ J.* 2015, 79, 1470-1478.

35 Calder, P. C., The role of marine omega-3 (n-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability. *Mol. Nutr. Food Res.* 2012, 56, 1073-1080.

36 Viola, J. R., Lemnitzer, P., Jansen, Y., Csaba, G., Winter, C., Neideck, C., Silvestre-Roig, C., Dittmar, G., Döring, Y., Drechsler, M., Weber, C., Zimmer, R., Cenac, N., and Soehnlein, O. Resolving lipid mediators maresin 1 and resolvin D2 prevent atheroprogession in mice. *Circ. Res.* 2016, 119, 1030-1038.

37 Salic, K., Morrison, M. C., Verschuren, L., Wielinga, P. Y., Wu, L., Kleemann, R., Gjorstrup, P., and Kooistra, T., Resolvin E1 attenuates atherosclerosis in absence of cholesterol-lowering effects and on top of atorvastatin. *Atherosclerosis.* 2016, 250, 158-165.

38 Oh, D. Y., Walenta, E., Akiyama, T. E., Lagakos, W. S., Lackey, D., Pessentheiner, A. R., Sasik, R., Hah, N., Chi, T. J., Cox, J. M., Powels, M. A., Di Salvo, J., Sinz, C., Watkins, S. M., Armando, A. M., Chung, H., Evans, R. M., Quehenberger,

O., McNelis, J., Bogner-Strauss, J. G., and Olefsky, J. M. A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice. *Nat Med.* 2014, 20, 942-947.

39 Shewale, S. V., Brown, A. L., Bi, X., Boudyguina, E., Sawyer, J. K., Alexander-Miller, M. A., and Parks, J. S., *In vivo* activation of leukocyte GPR120/FFAR4 by PUFAs has minimal impact on atherosclerosis in LDL receptor knockout mice. *J. Lipid Res.* 2017, 58, 236-246.

40 Anon., Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet.* 1999, 354, 447-455.

41 Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y., Saito, Y., Ishikawa, Y., Oikawa, S., Sasaki, J., Hishida, H., Itakura, H., Kita, T., Kitabatake, A., Nakaya, N., Sakata, T., Shimada, K., Shirato, K; Japan EPA lipid intervention study (JELIS) Investigators. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet.* 2007, 369, 1090-1098.

42 Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M. G., Geraci, E., Levantesi, G., Maggioni, A. P., Mantini, L., Marfisi, R. M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G. L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C., Valagussa, F; GISSI-Prevenzione Investigators. *Circulation* 2002, 105, 1897-1903.

43 Franzese, C. J., Bliden, K. P., Gesheff, M. G., Pandya, S., Guyer, K. E., Singla, A., Tantry, U. S., Toth, P. P., and Gurber, P. A. Relation of fish oil supplementation to

markers of atherothrombotic risk in patients with cardiovascular disease not receiving lipid-lowering therapy. *Am. J. Cardiol.* 2015, *115*, 1204-1211.

44 Rauch, B., Schiele, R., Schneider, S., Diller, F., Victor, N., Gohlke, H., Gottwik, M., Steinbeck, G., Del Castillo, U., Sack, R., Worth, H., Katus, H., Spitzer, W., Sabin, G., Senges J; OMEGA Study Group. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. *Circulation.* 2010, *122*, 2152-2159.

45 Bosch, J., Gerstein, H. C., Dagenais, G. R., Díaz, R., Dyal, L., Jung, H., Maggiono, A. P., Probstfield, J., Ramachandran, A., Riddle, M. C., Rydén, L. E., Yusuf, S; ORIGIN Trial Investigators. n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. *N. Engl. J. Med.* 2012, *367*, 309-318.

46 Aung, T., Halsey, J., Kromhout, D., Gerstein, H. C., Marchioli, R., Tavazzi, L., Geleijnse, J. M., Rauch, B., Ness, A., Galan, P., Chew, E. Y., Bosch, J., Collins, R., Lewington, S., Armitage, J., Clarke, R; Omega-3 Treatment Trialists' Collaboration. Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77 917 individuals. *JAMA Cardiol.* 2018, *3*, 225-234.

47 Alexander, D. D., Miller, P. E., Van Elswyk, M. E., Kuratko, C. N., and Bylsma, L. C., A meta-analysis of randomized controlled trials and prospective cohort studies of eicosapentaenoic and docosahexaenoic long-chain omega-3 fatty acids and coronary heart disease risk. *Mayo Clin. Proc.* 2017, *92*, 15-29.

48 Maki, K. C., Palacios, O. M., Bell, M., and Toth, P. P., Use of supplemental long-chain omega-3 fatty acids and risk for cardiac death: An updated meta-analysis and review of research gaps. *J. Clin. Lipidol.* 2017, *11*, 1152-1160.

- 49 Sekikawa, A., Doyle, M. F., and Kuller, L. H., 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 Cardiovasc. Med.* 2015, 25, 717-723.
- 50 Niki, T., Wakatsuki, T., Yamaguchi, K., Taketani, Y., Oeduka, H., Kusunose, K., Ise, T., Iwase, T., Yamada, H., Soeki, T., and Sata, M., Effects of the addition of eicosapentaenoic acid to strong statin therapy on inflammatory cytokines and coronary plaque components assessed by integrated backscatter intravascular ultrasound., *Circ. J.* 2016, 80, 450-460.
- 51 Watanabe, T., Ando, K., Daidoji, H., Otaki, Y., Sugawara, S., Matsui, M., Ikeno, E., Hirono, O., Miyawaki, H., Yashiro, Y., Nishiyama, S., Arimoto, T., Takahashi, H., Shishido, T., Miyashita, T., Miyamoto, T., Kubota, I; CHERRY study investigators., A randomized controlled trial of eicosapentaenoic acid in patients with coronary heart disease on statins. *J. Cardiol.* 2017, 70, 537-544.
- 52 Alfaddagh, A., Elajami, T. K., Ashfaq, H., Saleh, M., Bistran, B. R., and Welty, F. K., Effect of eicosapentaenoic and docosahexaenoic acids added to statin therapy on coronary artery plaque in patients with coronary artery disease: A randomized clinical trial., *J. Am. Heart Assoc.*, 2017, 15, 6.
- 53 Nosaka, K., Miyoshi, T., Iwamoto, M., Kajiya, M., Okawa, K., Tsukuda, S., Yokohama, F., Sogo, M., Nishibe, T., Matsuo, N., Hirohata, S., Ito, H., and Doi, M., Early initiation of eicosapentaenoic acid and statin treatment is associated with better clinical outcomes than statin alone in patients with acute coronary syndromes: 1-year outcomes of a randomized controlled study., *Int. J. Cardiol.*, 2017, 228, 173-179.
- 54 Bhatt, D. L., Steg, P. G., Brinton, E. A., Jacobson, T. A., Miller, M., Tardif, J. C., Ketchum, S. B., Doyle, R. T. Jr., Murphy, S. A., Soni, P. N., Braeckman, R. A., Juliano,

R. A., Ballantyne, C. M; REDUCE-IT Investigators. Rationale and design of REDUCE-IT: Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial. *Clin. Cardiol.* 2017, 40, 138-148.

55 Davidson, M. H., and Benes, L. B., The future of n-3 polyunsaturated fatty acid therapy. *Curr. Opin. Lipidol.* 2016, 27, 570-578.

56 Grenon, S. M., Aguado-Zuniga, J., Hatton, J. P., Owens, C. D., Conte, M. S., and Hughes-Fulford, M., Effects of fatty acids on endothelial cells: inflammation and monocyte adhesion. *J. Surg. Res.* 2012, 177, e35-43.

57 Bousserouel, S., Brouillet, A., Béréziat, G., Raymondjean, M., and Andréani, M., Different effects of n-6 and n-3 polyunsaturated fatty acids on the activation of rat smooth muscle cells by interleukin-1 beta. *J. Lipid Res.* 2003, 44, 601-611.

58 Pietsch, A., Weber, C., Goretzki, M., Weber, P. C., and Lorenz, R. L., N-3 but not N-6 fatty acids reduce the expression of the combined adhesion and scavenger receptor CD36 in human monocytic cells. *Cell. Biochem. Funct.* 1995, 13, 211-216.

59 Griffin, B. A., How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. *Curr. Opin. Lipidol.* 2008, 19, 57-62.

60 Khandelwal, S., Kelly, L., Malik, R., Prabhakaran, D., and Reddy, S., Impact of omega-6 fatty acids on cardiovascular outcomes: A review. *J. Preventive Cardiol.* 2013, 2, 325-336.

61 Yamashita, T., Oda, E., Sano, T., Ijiru, Y., Giddings, J. C., and Yamamoto, J., Varying the ratio of dietary n-6/n-3 polyunsaturated fatty acid alters the tendency to thrombosis and progress of atherosclerosis in apoE^{-/-} LDLR^{-/-} double knockout mouse. *Thromb. Res.* 2005, 116, 393-401.

- 62 Zhang, L., Geng, Y., Yin, M., Mao, L., Zhang, S., and Pan, J., Low omega-6/omega-3 polyunsaturated fatty acid ratios reduce hepatic C-reactive protein expression in apolipoprotein E-null mice. *Nutrition*. 2010, 26, 829-834.
- 63 Wang, S., Wu, D., Matthan, N. R., Lamon-Fava, S., Lecker, J. L., and Lichtenstein, A. H., Reduction in dietary omega-6 polyunsaturated fatty acids: eicosapentaenoic acid plus docosahexaenoic acid ratio minimizes atherosclerotic lesion formation and inflammatory response in the LDL receptor null mouse. *Atherosclerosis*. 2009, 204, 147-155.
- 64 Wan, J. B., Huang, L. L., Rong, R., Tan, R., Wang, J., and Kang, J. X., Endogenously decreasing tissue n-6/n-3 fatty acid ratio reduces atherosclerotic lesions in apolipoprotein E-deficient mice by inhibiting systemic and vascular inflammation. *Arterioscler. Thromb. Vasc. Biol.* 2010, 30, 2487-2494.
- 65 Fan, Y. Y., Ramos, K. S., and Chapkin, R. S., Dietary gamma-linolenic acid suppresses aortic smooth muscle cell proliferation and modifies atherosclerotic lesions in apolipoprotein E knockout mice. *J Nutr.* 2001, 131, 1675-1681.
- 66 Takai, S., Jin, D., Kawashima, H., Kimura, M., Shiraishi-Tateishi, A., Tanaka, T., Kakutani, S., Tanaka, K., Kiso, Y., and Miyazaki, M., Anti-atherosclerotic effects of dihomo-gamma-linolenic acid in ApoE-deficient mice. *J. Atheroscler. Thromb.* 2009, 16, 480-489.
- 67 Sanders, T. A., Lewis, F., Slaughter, S., Griffin, B. A., Griffin, M., Davies, I., Millward, D. J., Cooper, J. A., and Miller, G. J., Effect of varying the ratio of n-6 to n-3 fatty acids by increasing the dietary intake of alpha-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45-70 y: the OPTILIP study. *Am. J. Clin. Nutr.* 2016, 84, 513-522.

- 68 Griffin, M. D., Sanders, T. A., Davies, I. G., Morgan, L. M., Millward, D. J., Lewis, F., Slaughter, S., Cooper, J. A., Miller, G. J., and Griffin, B. A., Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. *Am. J. Clin. Nutr.* 2006, 84, 1290-1298.
- 69 Jakobsen, M. U., O'Reilly, E. J., Heitmann, B. L., Pereira, M. A., Bälter, K., Fraser, G. E., Goldbourt, U., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens, J., Virtamo, J., Willett, W. C., and Ascherio, A., Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies., *Am. J. Clin. Nutrition* 2009, 89, 1425-1432.
- 70 Katan, M. B., Omega-6 polyunsaturated fatty acids and coronary heart disease. *Am. J. Clin. Nutr.* 2009, 89, 1283-1284.
- 71 Mozaffarian, D., Micha, R., and 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, 23, 7.
- 72 Hamley, S., The effect of replacing saturated fat with mostly n-6 polyunsaturated fat on coronary heart disease: a meta-analysis of randomised controlled trials. *Nutr J.* 2017, 16, 30.
- 73 Chowdhury, R., Warnakula, S., Kunutsor, S., Crowe, F., Ward, H. A., Johnson, L., Franco, O. H., Butterworth, A. S., Forouhi, N. G., Thompson, S. G., Khaw, K. T., Mozaffarian, D., Danesh, J., and Do Angelantonio, E., Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann. Intern. Med.* 2014, 160, 398-406.

- 74 Oh, K., Hu, F. B., Manson, J. E., Stampfer, M. J., and Willett, W. C., Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. *Am. J. Epidemiol.* 2005, *161*, 672-679.
- 75 Farvid, M. S., Ding, M., Pan, A., Sun, Q., Chiuve, S. E., Steffen, L. M., Willett, W. C., and Hu, F. B., Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies. *Circulation* 2014, *130*, 1568-1578.
- 76 Laaksonen, D. E., Nyssönen, K., Niskanen, L., Rissanen, T. H., and Salonen, J. T., Prediction of cardiovascular mortality in middle-aged men by dietary and serum linoleic and polyunsaturated fatty acids. *Arch. Intern. Med.* 2005, *165*, 193-199.
- 77 Tomiyama, H., Matsumoto, C., Odaira, M., Yamada, J., Yoshida, M., Shiina, K., Nagata, M., and Yamashina, A., Relationships among the serum omega fatty acid levels, serum C-reactive protein levels and arterial stiffness/wave reflection in Japanese men. *Atherosclerosis.* 2011, *217*, 433-436.

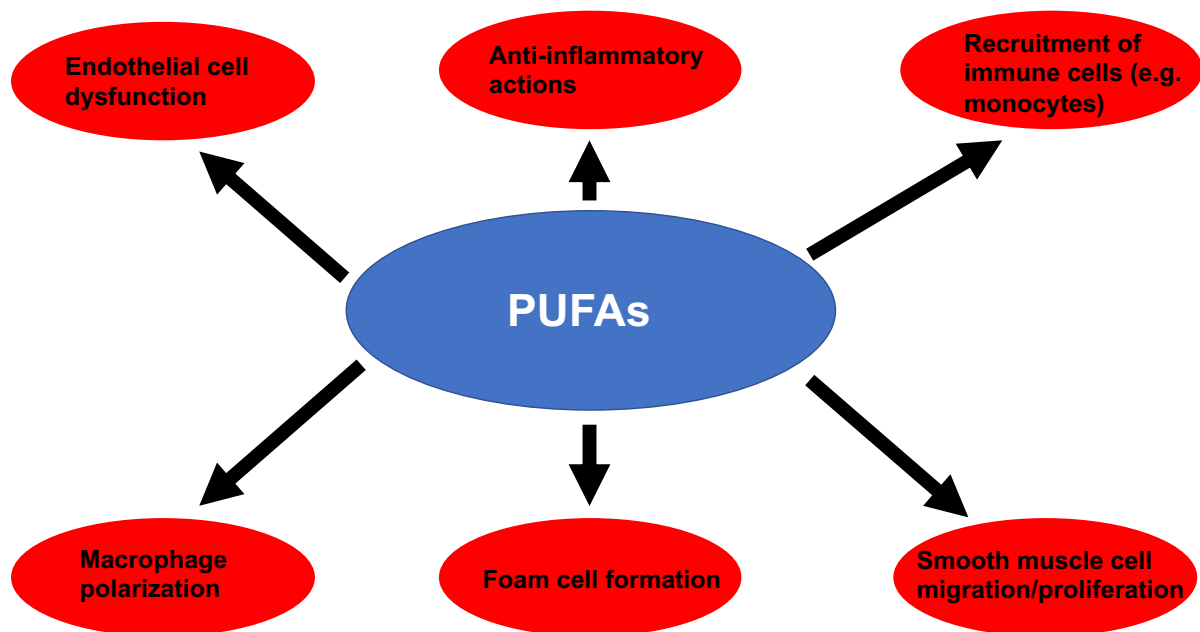


Figure 1. Omega-3 and -6 PUFAs exert their actions on multiple steps involved in atherosclerosis

See text for details.