Lipids and cardiovascular disease: where does dietary intervention sit alongside statin therapy?
Megson, Ian; Zabetakis, Ioannis; Whitfield, Phillip D

Published in:
Food Funct.
Publication date:
2016
Publisher rights:
http://www.rsc.org/journals-books-databases/journal-authors-reviewers/processes-policies/#accepted-manuscripts
Deposit the accepted version of the submitted article in their institutional repository(ies). There shall be an embargo of 12 months from the date of acceptance, after which time the article will be made available to the public. There shall be a link from this article to the PDF of the version of record on the Royal Society of Chemistry’s website, once this final version is available.
The re-use license for this item is:
CC BY-NC
The Document Version you have downloaded here is:
Early version, also known as pre-print

The final published version is available direct from the publisher website at:
10.1039/C6FO00024J

Link to author version on UHI Research Database

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the UHI Research Database are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights:

1) Users may download and print one copy of any publication from the UHI Research Database for the purpose of private study or research.
2) You may not further distribute the material or use it for any profit-making activity or commercial gain
3) You may freely distribute the URL identifying the publication in the UHI Research Database

Take down policy
If you believe that this document breaches copyright please contact us at RO@uhi.ac.uk providing details; we will remove access to the work immediately and investigate your claim.

Download date: 02. Aug. 2023
This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Lipids and cardiovascular disease: where does dietary intervention sit alongside statin therapy?

Ian L Megson\textsuperscript{a}, Phillip D Whitfield\textsuperscript{a} and Ioannis Zabetakis\textsuperscript{b}

The Seven Countries Study suggested an association between serum cholesterol and cardiovascular disease (CVD). However, the association was not consistent across the various cohorts of participants in different countries; while it was very clear in US and Northern European cohorts, it was weak in Southern European and Japanese cohorts. Nevertheless, the study triggered research into cholesterol-lowering drug strategies, ultimately leading to the development of statins amongst others. Clinical evidence in support of statins is strong and the vast majority of the medical community advocate these drugs as highly effective first-line therapeutics in primary and secondary prevention of CVD. However, growing evidence of side-effects associated with statins in a significant proportion of patients suggests that these drugs are not a universal solution to CVD.

There is a need, therefore, to revisit the evidence and to re-appraise the relative importance of cholesterol amongst many other lipids as potential modulators of atherogenesis. In this review, we assess the relative merits of statin therapy in CVD versus dietary interventions that impact on lipids other than cholesterol, including omega-3 fatty acids and polar lipid fractions of various foods (e.g. fish and olive oil). We conclude that careful design around the lipid components of dietary interventions presents a credible alternative in patients who are intolerant to statins or averse to taking such drugs.
**Introduction**

**Cardiovascular Disease**

Atherosclerosis is the complex process (Figure 1) that underpins the clinically relevant manifestations of cardiovascular disease, including ischaemic stroke, myocardial infarction and peripheral vascular disease. It has long been recognised that atheroma in humans is composed primarily of lipid. Atherosclerosis involves the accumulation of cholesterol, cholesterol esters, triglycerides and phospholipids in conduit arterial walls, initially forming fatty streaks and progressing to complex atherosclerotic plaques that partially occlude the lumen of the vessel and restrict blood flow. Plaque development often resolves to leave a lipid-rich protuberance in the vessel wall, overlain by smooth muscle and devoid of ongoing inflammation. These “stable” plaques tend not to present a threat to life, although they can cause debilitating symptoms if they cause a substantial reduction in blood flow. A majority of plaques, however, persist in a complex, unstable state which is prone to erosion and/or rupture. Plaque rupture exposes the pro-thrombotic core of the plaque to the blood and results in the rapid development of a thrombus in situ, which can occlude the artery and result in a myocardial infarction or ischaemic stroke, depending on the location of the thrombus.

**Figure 1**

**Evidence for the lipid hypothesis**

Several population-based studies were conducted in the mid-to-late twentieth century, the findings of which supported an association between blood-borne cholesterol (serum total cholesterol) and cardiovascular risk. The so-called “lipid hypothesis” was proposed in the late 1970s, stating that lowering blood cholesterol would be an effective means of reducing cardiovascular risk. The lipid hypothesis is widely accepted amongst the medical community, but is still hotly disputed by a minority on the grounds of flawed methodology and publication bias.

Part of the problem with the lipid hypothesis is that, in light of more recently accumulated knowledge of atherosclerosis, it is over-simplistic, both in its definition of “lipid” and in its interpretation of the role of lipids in the atherogenic process. In the context of the lipid hypothesis, “lipid” really refers to “total serum cholesterol”, to the exclusion of other important lipid classes and with little consideration of the blood-borne lipid transport particles (chylomicrons, VLDL, LDL, IDL and HDL). In addition, lipid accumulation in the vessel wall is an inflammatory process with a requirement for oxidative modification of cholesterol to progress. Targeted anti-inflammatory agents and antioxidants might, therefore, have as much of a therapeutic role to play as cholesterol lowering in slowing the disease process. Indeed, given our current understanding of the principal role played by inflammation in plaque stability, selective anti-inflammatory agents might represent the best target for preventing plaque rupture, although no anti-inflammatory interventions have yet shown clinical benefit in this setting.

**Pharmaceutical Interventions for CVD**

The high incidence and mortality associated with atherosclerosis makes it a major target for pharmaceutical intervention. The complex nature of the disease promotes a number of potential drug targets, from oxidative stress to inflammation, thrombosis and lipid lowering. By far the most successful target to date has been lowering of circulating cholesterol. Statins are by far the best known and most frequently used drugs to lower cholesterol and, as such, are the focus of this review. However, it is important to note that other cholesterol-lowering strategies have emerged with alternative mechanisms to reduce cholesterol, most notably inhibitors of dietary cholesterol absorption (stanols, sterols and the drug ezetimide), and propionate convertase subtilisin/kexin type 9 serine protease (PCSK9) inhibitors, which are now approved to be used as adjuvants to statins in high risk patients (e.g. with familial hypercholesterolaemia) by modulating LDL receptor degradation.

**Cholesterol-lowering by statins**

Besides dietary intervention studies, the other major consequence of the Seven Countries Study was the triggering of pharmaceutical industry interest in therapeutic interventions to lower cholesterol. Notwithstanding the controversy around the lipid hypothesis, cholesterol-lowering was considered to be a therapeutic target worth exploring. At the outset, it was recognised that the major determinant of blood-borne cholesterol was de novo synthesis in the liver; dietary intake is a secondary, but nevertheless modifiable, contributor. 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase is known to be the rate-limiting step in the synthetic pathway and was first targeted in the 1970s. The pharmaceutical promise of this approach was quickly recognised, leading to the development of the first commercially available statin, lovastatin. Since then, a range of statins have been developed by different pharmaceutical companies, each with subtle differences in terms of benefits and side-effect profile.

**Biochemistry**

While the concept of inhibiting the rate-limiting enzyme involved in hepatic cholesterol synthesis is straightforward, the means by which the inhibition effects reduced cholesterol levels in the blood is more complex than might have been envisaged at the outset (Figure 2). Certainly, the reduced synthesis manifests in a reduction in export of cholesterol from hepatocytes in the form of VLDL. However, low intracellular concentrations of cholesterol also induce an adaptive response whereby expression of LDL receptors (LDL-Rs) is enhanced to increase sequestration of cholesterol-laden LDL from the blood, presumably in order to maintain intracellular
cholesterol stocks. In addition, it appears that statins have a significant impact on high density lipoprotein (HDL),\(^{30,31}\) which could contribute to the antiatherogenic effects of statins by increasing so-called reverse cholesterol transport - out of LDL-engorged macrophages (foam cells) and back to the liver. Evidence relating to atorvastatin suggests that the mechanism underpinning this effect might be enhanced Apo-A1 production and subsequent elevation of the plasma HDL pool.\(^{32}\) Disentangling the relative benefits of increased plasma HDL vs. reduced plasma LDL is all but impossible, but it is widely accepted that the dramatic improvement in HDL:LDL ratio is the principal driver for improved outcome associated with statins in patients with cardiovascular disease.

Figure 2

**Pleiotropic effects**

Another unforeseen benefit of statins is that they might confer benefit through mechanisms not associated with cholesterol trafficking – so-called pleiotropic effects.\(^{33}\) Interestingly, many of the pleiotropic effects described to date relate to protection against endothelial dysfunction, thrombosis, inhibition of inflammation and/or oxidative stress,\(^{37,38}\) suggesting that statins might strike at all of the key processes involved in atherogenesis (Figure 2), often through mechanisms not directly mediated by cholesterol.\(^{39}\) Amongst the most convincing of the pleiotropic effects described to date are anti-inflammatory and antioxidant effects.\(^{37,38}\)

**Clinical Evidence: Benefits of Statins in cardiovascular disease**

Over the past 25 years, a number of clinical studies have been conducted to assess the ability of statins to reduce cardiovascular risk. A meta-analysis of 14 randomised trials (90,056 patients) found that statins effectively reduced the risk of major vascular events (fatal and non-fatal) by ~20% per mM LDL cholesterol reduction. This translated into 4.8% fewer vascular events in patients with pre-existing CHD and 2.5% in those without a history of CHD.\(^{40}\) A meta-analysis of intensive lipid-lowering concurred with ~20% reduction per mM LDL cholesterol lowering and concluded that lowering cholesterol by 2-3 mM could reduce risk by 40-50%.\(^{41}\) The Cochrane Review of eighteen randomised control trials (56,934 participants) found a combined fatal and non-fatal CVD relative risk of 0.75 (95% CI 0.70 to 0.81), combined fatal and non-fatal CHD events relative risk of 0.73 (95% CI 0.67 to 0.80) and combined fatal and non-fatal stroke relative risk of 0.78 (95% CI 0.68 to 0.89).\(^{42}\) A separate systematic review compared studies with three different statins (atorvastatin, pravastatin and simvastatin) and found there to be no significant difference between them in terms of risk reduction.\(^{43}\) The latest finding relates back to one of the earliest statin trials - the West of Scotland Coronary Prevention Study (WOSCOPS) - which shows a legacy benefit of statin treatment at 15 years after the 5 year study, amounting to a 21% reduction in cardiovascular mortality.\(^{44}\) Indeed, the effect of statins is sufficiently powerful to skew the findings of contemporary observational studies.\(^{45}\)

On the face of it, these data are incontrovertible: statins reduce cardiovascular risk and the more intensive the better. However, there remains a surprisingly vociferous lobby, including clinicians, scientists and patients, which is vehemently against widespread statin use.\(^{46}\) While some of the scepticism might hark back to perceived flaws in the original lipid hypothesis and the early epidemiological data, some reflects genuine concerns about study design, reporting bias and underestimation of side effects.

**Side effects**

The HMG-CoA pathway is not only responsible for cholesterol synthesis, but also several other cellular processes, including prenylation (linking of lipid moieties to aid association with cell membranes) of specific proteins. Inhibition of this process is believed to have both beneficial and detrimental effects: many of the pleiotropic effects of statins are attributed, at least in part, to inhibition of prenylation, as are some of the reductions in cancer risk. However, inhibition of prenylation has also been implicated in some of the adverse effects of statins.

Meta-analyses have found no evidence to suggest that statins have any adverse impact on cancer incidence; indeed statins may even reduce the risk of certain cancers (oesophageal, colo-rectal, gastric and prostate). However, statins are known to increase the risk of liver damage,\(^{47}\) and, ironically, to increase risk of type 2 diabetes,\(^{47,48}\) one of the major risk factors for cardiovascular-related deaths. The headline figure for increased risk of type 2 diabetes amongst patients on statins in the METSIM trial was 46%, with a 24% decrease in insulin sensitivity and 12% reduction in insulin secretion.\(^{48}\) Importantly, for two of the most prescribed statins, simvastatin and atorvastatin, the effect was dose-dependent, which presents a counter-argument to using increased statin doses to keep LDL cholesterol levels as low as possible. Mild cognitive impairment, neuropathy and sexual dysfunction are also weakly associated with statin use.

Perhaps the most commonly reported side-effect of statins (in up to 15% of patients receiving statins), however, relate to muscle damage and pain (myopathy and myalgia).\(^{49,50}\) Whilst not life-threatening, these effects can be debilitating and have a substantial impact on quality of life and, in some cases, result in long-term muscle damage. It is these side-effects that are primarily responsible for patient intolerance and non-compliance to statin therapies and should not be underestimated.

**Lipid-based dietary interventions for CVD**

Given the importance of a range of lipids at different points in the atherosclerotic process, it is unsurprising that dietary interventions have long been advocated as a potential preventative measure or...
intervention in CVD. The most obvious such intervention is low fat and low cholesterol diets and ingredients, but for the purposes of this review we will concentrate on supplementation of lipid moieties that are considered to be beneficial in the CVD setting.

**Omega-3 polyunsaturated fatty acids**

The emphasis from the lipid hypothesis-driven research was centred on lipids (or more accurately, cholesterol) being harmful, but at the same time, there was significant evidence building to suggest that another class of lipids, Omega-3 polyunsaturated fatty acids, might actually be beneficial. Epidemiological studies indicated that certain populations and cultures (e.g. Netherlands, Finland, Mediterranean, Greenland Inuit and Danes, Japan) have notably lower incidence of CVD than others. A common feature of the diet amongst these populations is a relatively high dietary intake of fish. Irrespective of the many shortcomings (highlighted in the research that identified fish as one of several mediators of the effects seen, the association between fish, and oily fish in particular, with cardiovascular disease is upheld in meta-analyses and has fuelled a huge research interest in the concept that eating fish might hold health benefits. There is now substantial evidence to support the benefits of dietary consumption of fish, particularly oily varieties such as salmon, trout, sardines, mackerel and herring, to help combat cardiovascular disease. The paradigm for the beneficial effects relating to these fish is that they are high in polyunsaturated fatty acids (PUFAs) and omega-3 fatty acids in particular. Although the merits of omega-3 fatty acids are sometimes tied to their antioxidant activity, it is clear that their activity is complex and multi-factorial. An important mode of action is via provision of substrate for synthesis of inflammation-resolving eicosanoids (resolvins, protectins and maresins), but they are also recognised to reduce triglyceride levels, to have anti-thrombotic effects, increase membrane fluidity and to induce plaque stability; many of these effects are less well understood. In the wake of early highly-publicised findings, health providers in many countries issued guidelines in support of eating oily fish and/or taking omega-3 supplements. However, once blinded studies were conducted in patient groups with already optimised conventional therapy (e.g. statins), much of the euphoria subsided and some health authorities retracted their advice on pure omega-3 supplements, but maintained their support for eating oily fish. Based on the current evidence, the National Institute for Health & Care Excellence guidelines in UK recommends: “that people at high risk of, or with, cardiovascular disease should be advised to consume at least two portions of fish per week, including a portion of oily fish. However it advises that omega-3 fatty acid compounds should not be offered for the prevention of cardiovascular disease to people who are being treated for primary or secondary prevention of cardiovascular disease, alone or in combination with a statin, including in people with chronic kidney disease or type 1 or type 2 diabetes.”

Omega 3 is not licensed for use post-myocardial infarction (MI) in the USA, but the American Heart Association does recommend the use of oily fish or omega-3 capsules post-MI. Meanwhile, the European Cardiology Society notes the lack of evidence to support omega-3 use in secondary prevention (e.g. post-MI), but advocates its use in reducing triglycerides as an adjunct to statins.

The dichotomy in advice between whole oily fish and omega-3 products is striking and suggests that there is a sense in the clinical community that omega-3 is not yet sufficiently proven to justify the costs of prescription – advising people to eat oily fish does not carry the same cost to healthcare providers and, arguably, is supported by better evidence. Despite the discrepancy between findings from whole fish or whole fish oil and those from pure omega-3s, little attention has been given to understanding the dichotomy. One obvious explanation is that whole fish/whole fish oil is a complex mixture of lipids, only a small component of which is omega-3. There is potential, therefore, for other lipids in the mixture to be beneficial, either in an additive or even a synergistic way.

**Fish/fish oil/omega-3 consumption and cardiovascular disease (coronary artery disease and its consequences)**

By way of a very brief review of the clinical studies relating to oily fish and omega-3 capsules, the clinical data can be broadly divided into population-based studies and randomized control trials.

**Population-based studies**

Numerous population-based studies have been conducted over the past 40 years to establish an association between fish-eating dietary habits and risk of coronary artery disease and cardiovascular-related sudden death. As always, given the heterogeneity of the studies conducted, with different endpoints, populations, average fish consumption and type of fish consumed, it is difficult to reach firm conclusions regarding the association, but meta-analyses of these studies would suggest that there is an association between fish consumption and risk of coronary artery disease and myocardial infarction (MI). There is always a danger, however, of over-interpreting an association as a causal relationship, so these findings should really only be seen as a fore-runner for randomised controlled trials to test the hypothesis robustly.

**Randomized trials**

A wide range of trials have reported on the impact of fish, fish oils and omega-3 capsules on risk of cardiovascular disease. The first two trials of this kind were GISSI-P (1999) and JELIS (2007), a significant relative risk reduction (~20-35% depending on outcome measure) was found for both trials but, importantly, these were open-label trials and did not have a placebo control arm. Four further trials reported in 2007 (GISSI-HF) and 2010 (Alpha Omega and SU.FOL.OM3). GISSI-HF found a modest but significant benefit of omega-3 (~8-10% risk reduction), whereas none of the three other trials showed any benefit. The reason for the difference in the outcome of GISSI-HF is not easy to identify, but it might be important that the target patient group was...
heart failure, rather than CAD or post-MI, as for the other 3 trials. These trials are summarised in Table 1. It is important to highlight, here, the fact that the placebo used in the Omega study was olive oil. Given the strong antioxidant and anti-inflammatory activities of olive oil, choosing this placebo is a true limitation of the Omega study.

Fish/fish oil/omega-3 consumption and blood pressure

A few early studies investigated the impact of fish vs red meat on blood pressure, with mixed results. A number of studies have investigated the impact of fish oil supplements on blood pressure in both patients with hypertension (therapeutic) or normotensive people (preventative). Interestingly, supplementation in these studies is generally quantified in terms of EPA+DHA — indicative of the importance assigned to omega-3s in these studies. Meta-analyses of the best designed studies indicate a modest (~2 mm Hg) fall in blood pressure amongst patients, but little or no effect in normotensive individuals (which is not necessarily a bad thing).67, 68 There was no correlation between supplement fish oil dose, and effect across these trials. However, a previous meta-analysis had indicated an association between omega-3 dose and effect.

Taken together, it is perhaps easy to understand why the clinical community is convinced by the argument that eating oily fish might hold benefit (based on the population studies, with all their limitations), but that omega-3 capsules might not (based on randomized placebo-controlled trials). If oily dietary fish really does have improved benefit versus Omega-3 capsules, the inference is that other components of oily fish are important beyond omega-3.

Table 1. Double-blind intervention studies on the cardioprotective impact of statins and omega-3 polyunsaturated fatty acids.

<table>
<thead>
<tr>
<th>Trial title</th>
<th>Type of intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>GISSI-P61</td>
<td>Intervention (4 groups: n-PUFA, n-PUFA and vitamin E, vitamin E, placebo-control)</td>
<td>Treatment with n-3 PUFA, but not vitamin E, significantly lowered the risk of death and cardiovascular death</td>
</tr>
<tr>
<td>JELIS62</td>
<td>Intervention [2 groups: EPA and statin, control (statin only)]</td>
<td>EPA and statin lead to a 19% relative reduction in major coronary events</td>
</tr>
<tr>
<td>GISSI-HF63</td>
<td>Intervention (2 groups: rosuvastatin, placebo-control)</td>
<td>Statin treatment did not affect clinical outcomes in patients with chronic heart failure</td>
</tr>
<tr>
<td>Alpha Omega64</td>
<td>Intervention (4 groups: EPA-DHA, ALA, EPA-DHA and ALA, Supplementation with EPA-DHA or ALA did not significantly reduce the rate of major cardiovascular events</td>
<td></td>
</tr>
</tbody>
</table>

Mechanisms of action

Evidence as to the mode of action of omega-3 fatty acids has accumulated to support a number of different aspects of activity (Figure 3). Brief descriptions of the evidence in support of the major aspects are described below:

Figure 3

Anti-inflammatory effects

Omega-3 fatty acids have anti-inflammatory effects with the potential to influence both the atherogenic process and plaque stability.69 The most direct effect of DHA and EPA on inflammation is through substitution of arachidonic acid as substrate for cytochrome P<sub>450</sub> and LOX-mediated metabolism to generate different eicosanoid products with a pro-resolving rather than pro-inflammatory profile.70-72 The science around the resolvins, protectins and maresins is still fairly new, but they mediate the resolving phase of inflammation, whereas the arachidonic acid-derived equivalent eicosanoids perpetuate inflammation. From a cardiovascular perspective, this could have critical consequences with respect to plaque stability, where unresolved inflammation is recognised to be a risk factor for rupture.73-76 Fish consumption also has a beneficial impact on other inflammatory markers (CRP, IL-6, TNF-α) that have been associated with atherosclerosis,77 although it is not clear whether this is linked to the eicosanoid effects indicated above. In addition, a recent lipidomic profiling study in a mouse model has indicated that feeding mice with a DHA-enriched diet reduces AA-derived oxylipins and increases those related to both EPA and DHA.78 In addition, the best correlation was found in the model between atherosclerosis and liver F<sub>4</sub>-isoprostanes. Although causality was not tested, F<sub>4</sub>-isoprostanes might represent, at the least, a fish oil-related marker of cardiovascular health.

Inhibition of platelet aggregation

An inhibitory effect of dietary fish oils on platelet aggregation was one of the first suggested modes of action of omega-3s, with both in vitro and dietary intervention studies supporting an antiplatelet effect.79 From a mechanistic perspective,

This journal is © The Royal Society of Chemistry 20xx

J. Name., 2013, 00, 1-3 | 5

Please do not adjust margins
creditable data suggest an antagonistic effect of DHA and EPA at the TXA\textsubscript{2} receptor on platelets.\textsuperscript{80} However, in clinical trials fish oils have not shown a consistent effect on platelet aggregation or coagulation.\textsuperscript{58}

**PAF and food-derived polar lipids**

Platelet activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phospho-choline) was identified in 1979,\textsuperscript{91} and it orchestrates inflammation, thrombosis and oxidation.\textsuperscript{92} PAF is a crucial mediator in the inflammatory response and it can be biosynthesized by various cell types upon activation, e.g. platelets, monocytes, macrophages, foam cells and endothelial cells,\textsuperscript{93} while the levels of PAF levels are controlled by PAF-acetylhydrolases (PAF-AHs) which hydrolyze the sn-2 PAF group converting them to lyso-PAF.\textsuperscript{94} (see part 1: remodelling biosynthetic pathway of PAF in Figure 4).

Figure 4

PAF appears to belong to a family of lipids that have different structures and origins but share similar bioactivity.\textsuperscript{95} The PAF family includes lipids such as oxidatively fragmented phosphatidyl cholines that mimic the structure of PAF to serve as ligands for the PAF receptor; these lipids are called “PAF-like” lipids.\textsuperscript{96} The PAF-like activity of these lipids is due to a sn-2 residue that differs from that of PAF, which is exclusively derived from acetyl-CoA. As a consequence, PAF-like lipids that have been isolated from natural sources have totally different structures than PAF. The main differences are either at the sn-2 position, where instead of the acetyl group of PAF there is another two or four carbon residue or at the sn-3 position on the glycerol backbone, where these lipids have acetyl group(s) on sugar moiety(ies).\textsuperscript{95, 97} Other PAF-like lipids exert their bioactivity through two vicinal hydroxyl groups in the glyceryl ether carbon chain in the sn-1 position, for instance dihydroxyl-chimyl alcohol.\textsuperscript{100} Similarly, PAF antagonists have a wide range of structures: some are analogues of PAF (e.g. methoxy analogue of PAF), others, like the specific PAF receptors antagonists BN and WEB, are structurally different from PAF.\textsuperscript{92}

PAF is biosynthesized by two distinctive enzymatic pathways: a) the remodelling pathway catalyzed by lyso-PAF-acetyl-CoA acetyltransferase (Lyso-PAF-acetyltransferase; Lyso-PAF-AT, EC 2.3.1.67 which acetylates lyso-PAF (part 1 in Fig. 4)) and b) the de novo pathway which is catalyzed by a specific dithiothreitol-insensitive CDP-choline: 1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase (PAF-cholinephosphotransferase; PAF-CPT, EC. 2.7.8.16) that converts 1-O-alkyl-2-acetyl-glycerol to PAF (part 2 in Fig. 4). The catabolism of PAF, to its biologically inactive form, is catalyzed by a PAF specific acetylhydrolase (PAF-AH, EC 3.1.1.47) which plasma form is known as lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}) (part 3 in Figure 4).\textsuperscript{94}

There is a wealth of evidence that it is the lipid fraction of food that has clear in vitro, in vivo and ex vivo biological activities against inflammation and the onset of atherosclerosis.\textsuperscript{99} In a recent study, it was found that the polar lipid fraction of gilthead sea bream (Sparus aurata) modulated PAF metabolism in a rabbit model of atherosclerosis by down-regulating PAF biosynthesis (i.e. decreasing the activity of PAF-CPT) and up-regulating PAF catabolism (i.e. increasing the activity of Lp-PLA\textsubscript{2}).\textsuperscript{96} Several other studies have also found that polar lipids of principal food of the Mediterranean diet have clear anti-inflammatory activities.\textsuperscript{100} These studies provide strong evidence that the anti-inflammatory activity of the polar lipids is expressed by blocking the onset of inflammation without changing the levels of LDL-cholesterol in human blood,\textsuperscript{98} challenging the lipid hypothesis as the primary pathway for atherogenesis. Polar food lipids exert their beneficial actions by blocking the inflammation caused by PAF and these studies are presented in the next part of this paper.

**Anti-inflammatory activities of dietary polar lipids**

Given that PAF is recognised to be a potent pro-inflammatory mediator involved in the development of thrombosis and atherosclerosis, the presence of lipids in food that act as PAF antagonists is important in our quest to find alternative ways to inhibit atherogenesis. This inhibition is an indirect assessment tool for the cardioprotective properties of a specific food. There is a wide range of food of the Mediterranean diet that has been studied; these studies are summarised in Table 2.

Table 2: Platelet and inflammation studies on the cardioprotective impact of food polar lipids.

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar lipids of fish fed with olive pomace</td>
<td>抑制血小板聚集</td>
</tr>
<tr>
<td></td>
<td>In vitro study using washed rabbits’ platelets</td>
</tr>
<tr>
<td>Polar lipids of red and white wine</td>
<td>In vitro study using washed rabbits’ platelets</td>
</tr>
<tr>
<td>Polar lipids of olive oil</td>
<td>In vitro study using washed rabbits’ platelets</td>
</tr>
</tbody>
</table>

This journal is © The Royal Society of Chemistry 20xx

Food & Function
function and other pathological processes, including endothelial dysfunction, cholesterol peroxidation and inflammation. Epidemiological evidence pointing to an association between serum total cholesterol and CVD in some, but not all populations, was central to the lipid hypothesis that triggered research culminating in the development of statins and other cholesterol-lowering strategies that represent drug and functional food therapies for the prevention of CVD. However, the lipid hypothesis for CVD is not universally accepted and the surprisingly low CVD rate in Mediterranean countries, where the diet is relatively high in cholesterol, has never been fully explained. The same argument is the basis for the so-called "French-paradox", where the disproportionately low incidence of CVD mortality in light of smoking and dietary cholesterol is often attributed to the benefits of moderate red wine consumption. That there exist several examples of countries that do not support the lipid hypothesis for CVD (e.g. Mediterranean countries, Japan) at the very least merits a re-evaluation of the evidence. Does dietary cholesterol really represent the full story, or are there critical aspects of the Mediterranean diet that are at least as important as cholesterol content in determining the mortality rate in these countries?

In addressing these questions, it is important to highlight that not all fat is harmful: indeed, even cholesterol is not the pariah that it is often made out to be; after all, we synthesise it often made out to be; after all, we synthesise it.

All these studies have been carried out with a mixture of lipid molecules that can potentially have either pro-aggregatory or inhibitory activities. These activities depend on both the relative ability of each molecule to aggregate platelets or inhibit the PAF-induced platelet aggregation and on the relative amount of each molecule in the mixture/food. From this perspective, a fraction that aggregates platelets may also contain lipid molecules with inhibitory properties and vice versa. Lipids that act as PAF agonists in fact retard the inflammatory activities of PAF since they compete with PAF for the same receptors, effectively acting as antagonists to PAF, hence resulting in anti-inflammatory profiles.

In a mechanistic study performed by our research team, the in vivo effect of fish polar lipids in hypercholesterolaemic rabbits was linked to the in vitro effect that these lipids have on PAF biosynthetic and catabolic pathways. Healthy male New Zealand rabbits of specific weight were divided into two equal groups and were fed either normal rabbit food enriched with 1% cholesterol (atherogenic diet), or the atherogenic diet enriched with polar lipids of gilthead sea bream (0.06% w/w) for 45 days. Morphometric analysis of the arteries showed that atherosclerotic lesions in rabbits fed with diet enriched with fish polar were significantly less pronounced (76%, p<0.05) compared to those fed with the atherogenic diet. These results were associated with decreased PAF-CPT activity (see Figure 4) in leukocytes, as well as decreased activities of both PAF-CPT and Lyso PAF-AT in platelets of animals receiving polar lipids. In addition, it was found that both free and bound PAF levels increased in animals on the atherogenic diet alone, but decreased in those also receiving polar lipids (p<0.05). We suggest, therefore, that the supplementation of fish polar lipids to rabbits reduces the biosynthesis of PAF, which plays a role in limiting the atherosclerotic process in these animals.

Following this methodology based on the pivotal role of PAF in atherosclerosis and the cardioprotective role of PAF-inhibitors derived from olive pomace, the inclusion of olive pomace in fish feed has been carried out for the aquaculture production of gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax). Olive pomace inclusion in fish feed improved the nutritional value of both fish feed and fish possibly by enriching the marine lipid profile of gilthead sea bream (Sparus aurata) with specific bioactive lipid compounds of plant origin. The polar lipids of sea bass fed with an experimental diet containing olive pomace has been further studied. The most active fractions against platelet aggregation were further characterised by electrospray-mass spectrometry and it was elucidated that these lipid fractions contained various diacyl-glycerophospholipids species. The majority of these lipid species have either 18:0 or 18:1 fatty acids in the sn-1 position and either 22:6 or 20:2 fatty acids in the sn-2 position.

Conclusions

Atherosclerosis is a highly complex disease that requires the convergence of a number of pathological processes, including endothelial dysfunction, cholesterol peroxidation and inflammation. Therefore, the lipid hypothesis for CVD is not universally accepted and the surprisingly low CVD rate in Mediterranean countries, where the diet is relatively high in cholesterol, has never been fully explained. The same argument is the basis for the so-called "French-paradox", where the disproportionately low incidence of CVD mortality in light of smoking and dietary cholesterol is often attributed to the benefits of moderate red wine consumption. That there exist several examples of countries that do not support the lipid hypothesis for CVD (e.g. Mediterranean countries, Japan) at the very least merits a re-evaluation of the evidence. Does dietary cholesterol really represent the full story, or are there critical aspects of the Mediterranean diet that are at least as important as cholesterol content in determining the mortality rate in these countries?

In addressing these questions, it is important to highlight that not all fat is harmful: indeed, even cholesterol is not the pariah that it is often made out to be; after all, we synthesise it continuously in our livers. Add to this the recognition that fats from oily fish might offer some benefit, and the complex role of fats in CVD comes into focus. The key guiding principles should therefore be moderation and balance, with substantial inclusion of oily fish, fruit and vegetables, with a proportionate reduction in red meat and, importantly, sugar. While a reduction in dietary cholesterol might be one outcome of such a dietary intervention, it is not necessarily going to be the only contributor to any improvement in cardiovascular outcome that might accrue, given the potential impact on oxidative stress and inflammation that are also possible through other components of the diet.

Why not just statins?
While there is general consensus that statins have a substantial impact on cardiovascular mortality, there remains a question as to whether this is directly linked to depression of plasma LDL or whether it is also driven by the so-called pleiotropic effects attributed to these drugs (e.g. anti-inflammatory, antioxidant, HDL-enhancing properties). Furthermore, statins do not “cure” atherosclerosis, but instead slow the process: CVD is still a major killer despite widespread use of statins. In addition, there is growing evidence that, like all drugs, statins can induce debilitating, dose-dependent, side-effects in a small proportion of patients and should not therefore be considered to be a risk-free panacea to prevent CVD in everybody. This is an especially important consideration with respect to statins as preventative therapies, where the balance between risk and benefit is likely to be much less clearly in favour of benefit. Add the healthcare cost to this equation and the use of statins as a preventative measure is unappealing. In particular, should the increased risk of type 2 diabetes prove significant, there is a strong case that the benefits of statins might be offset by detrimental metabolic effects with substantial downstream health consequences.

**How can diet-derived lipids have a positive impact on atherosclerosis?**

Lipids are not universally harmful: after all, we actively synthesise cholesterol, fatty acids and triglycerides. Indeed, PUFAs from oily fish and plant sources are believed to be protective in CVD, although there is again some controversy as to whether PUFAs in isolation, or fish oils in general, are responsible for the benefits. The issue is further complicated by the results of several in vivo studies indicating that the development of atherosclerotic lesions independent of LDL-cholesterol and triglycerides in the blood, but was markedly influenced by the anti-inflammatory action of food polar lipids acting as PAF inhibitors. In fact, dietary polar lipids have a positive impact in several diseases, apparently without severe side effects since they were shown to reduce side effects of some drugs.16

It could be suggested that polar lipids are more efficient carriers of biological information since they are highly effective in delivering their fatty acid residues for incorporation into the membranes of cells involved in different biochemical phenomena and diseases, e.g. atherosclerosis or development of cancer. 16, 94 We postulate that beneficial effects of polar lipids that are abundant in Mediterranean diets might, alongside dietary antioxidant and fibre content, contribute to an explanation of some of the anomalies associated with the Seven Countries Study. Furthermore, the existence of alternative lipids from oily fish that might have cardiovascular benefits beyond omega-3 PUFAs might go some way to explaining why eating whole fish is the preferred advice for prevention of CVD amongst many clinicians. Polar lipids are, chemically, more agile - and therefore more bioactive - than omega-3 PUFAs that are administered as neutral esters that might undergo hydrolysis during absorption in the gut. It is likely, therefore, that the biochemical functionality of lipids is partially defined by their polarity, and not only the structure of the free fatty acid moieties. Also, the likely synergy between different components of a Mediterranean diet should perhaps indicate that we focus less on trying to identify specific components that convey benefit and instead accept that the whole is better than the sum of its parts.

**Where does dietary intervention sit alongside statin therapy for cardiovascular disease prevention?**

Notwithstanding the controversies that still exist relating to mechanism, the evidence supporting cardiovascular benefits for both statins and Mediterranean diets is strong. The choice as to which approach an individual should take to reduce risk is as much to do with personal perspective and attitude to drug interventions as it is to efficacy or intolerance. There is no doubt that, were we all willing and able to adopt the Mediterranean diet, our risk of cardiovascular disease would be reduced. That the populations in most developed countries have not adopted Mediterranean diets is testimony to how difficult it is to change behaviour. Equally, the fact that many individuals in Mediterranean countries who have always adhered to a healthy diet might require statins illustrates the point that, for some, dietary intervention is insufficient. Added to this, there is a small but perhaps growing population that would prefer to take a “natural” approach to disease prevention to the exclusion of therapies such as statins, while there is another group that are intolerant to statins for whom dietary intervention is more attractive because there are no recognised side-effects. There is clearly room, therefore, for both dietary and pharmaceutical approaches, either in isolation or in combination, to reducing cardiovascular risk – the decision as to which is best is as much a reflection of an individual’s view of disease self-management as it is of the evidence of efficacy of the two approaches.

**Authors’ contributions**

ILM and IZ contributed to the initial manuscript concept, the review and editing of the manuscript. PDW contributed to the revision.

**Acknowledgements**

Research in the Department of Diabetes & Cardiovascular Science at the University of the Highlands & Islands is supported in part by funds from Highlands & Islands Enterprise, Scottish Funding Council and European Regional Development Fund. We thank Prof Stephen Leslie (Cardiology, Raigmore Hospital, Inverness, UK), for valuable input to the clinical aspects of this manuscript.
References


Fig 1. Atherosclerosis: interaction of lipids and inflammation to generate foam cells in blood vessel walls.
Fig 2. Lipid transport and disposition. Site of impact of statins, cholesterol uptake inhibitors and dietary lipid control on the process. Key: ROS - reactive oxygen species; chol – cholesterol; -R - receptor; TRGs – triglycerides; FFAs – free fatty acids.
Fig 3. Proposed effects of Omega-3 fatty acids in risk reduction for cardiovascular disease.