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Review Article

Interaction between Statins and Omega 3 Poly-unsaturated Fatty Acids in Cardiovascular Disease: Synergistic or Not?

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ABSTRACT

Up to the middle of the 2000's, omega 3 polyunsaturated fatty acids were considered has having cardioprotective properties. Patients having a myocardial infarction were supplemented with these fatty acids in secondary prevention of myocardial infarction. Since then, many randomized clinical trials failed to observe the cardioprotective effects previously described. The main hypothesis to explain such change is the systematic prescription of statins to patients following a myocardial infarction, statins interfering with the homeostasis of omega 3 fatty acids. This review discusses the effects of different forms of omega-3 in association with statins on cardiovascular disease and emphasize on the interaction between statins and omega 3 fatty acids leading to the possible need to use higher dose of fatty acids to get a synergistic effect.

Introduction

The majority of randomized clinical trials (RCT) before the mids of the 2000's showed that ω3 polyunsaturated fatty acids (ω3-PUFAs) have beneficial effects especially in preventing a second myocardial infarction (MI). In 2002, based on the results of the GISSI-Prevenzione Trial, the American Heart Association (AHA) recommended to eat fish, mainly oily fishes, twice per week in the general population with a supplementation of 1g/day eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for people being coronaropathic [1]. Since then, such beneficial effects of ω3-PUFAs were controversial. The lack of omega-3 PUFAs' beneficial effects could be explained by some bias including the systematic prescription of statins to patients having a myocardial infarction [2, 3]. Statins are competitive inhibitors of HMG-CoA reductase, a transmembrane enzyme that is involved in the production of mevalonate, a pathway leading to the synthesis of cholesterol. Besides this well-known effect, statins also inhibit isoprenoids regulating transcription factors PPAR involved in the stabilization of the plaque and the regression of atheromatous lesions [4]. Thus, statins represent the first-line treatment in the control of cardiovascular events in dyslipidemic patients and their tolerance is rather acceptable when the indications are clearly exposed [5].LDL is the first target in the treatment of dyslipidemia. Current recommendations determine LDL levels to be less than 1 g/l for patients with high cardiovascular risk and less than 0.7 g/l for very high-risk patients. Six statins have a marketing authorization in Europe and the USA: fluvastatin, atorvastatin, rosuvastatin, lovastatin, pravastatin, and simvastatin. Even if a medical service has been obtained with statins, there is still a cardiovascular risk that persists. Moreover, statins have major adverse events such as myalgia, rhabdomyolysis, alteration of hepatic function and diabetes' risks. Before the arrival of statins, and after their use, ω3-PUFAs represented an adjuvant therapy in

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cardiovascular disease (CVD) [6]. Since the systematic prescription of statins to people having CVD, some studies found that ω3 PUFAs in neo-adjuvant therapy have no effects. For example, Sethi *et al.* did not find a reduction of the total mortality with ω3-PUFAs supplementation, especially when statins were prescribed [7]. In the opposite, recent studies underline that ω3 PUFAs might actually present some interests. In 2013, Otto *et al.* conducted a cohort of 2372 U.S. american citizens of different races (Caucasian, African, Hispanic, Asian) followed for 10 years and free of CVD [8]. They observed a strong cardioprotective effect associated with circulating EPA and DHA levels. This effect is however not found for all PUFAs including DPA, alpha-linoleic acid and AA.

Eussen *et al.* (2012) hypothesized that statins may reduce the protective effect of omega-3 fatty acids [9]. They observed that in the group of non-users of statins, only 9% of patients supplemented with ω3 fatty acid (400 mg EPA and DHA) developed cardiovascular complications, compared to 18% in the statin users group. Hence, they concluded that statins reduce the effect of ω3 PUFAs. Statins and ω3 PUFAs could, therefore, be counter-active at several levels and statins would appear to interfere with ω3 PUFAs homeostasis [10]. The lack of beneficial effects of ω3 PUFAs in some RCT might be due to bias [2-3]:

i. Dilution of the effects of ω3 PUFAs by the use of systematic multiple medication for patients having a cardiovascular high risk.
ii. The evaluation of the nutritional intake (generally neglected in the initial characteristics of patients) that can lead to a maximum effect of ω3 PUFAs before the use of supplements.
iii. The importance of a sufficient dose of supplementation to obtain an effect, especially in patients having a long history of cardiovascular problems.
iv. The importance of a sufficient duration of supplementation to get the effects (membrane incorporation, circulating levels, …).
v. The choice of ω3 PUFA: EPA and/or DHA?
vi. The inter-individual variability of PUFAs incorporation for a given supplementation.
vii. Given the efficiency of the statin used, the dose of ω3 PUFAs might be adapted to prevent possible interactions.
viii. The majority of negative RCT incorporated little sized population of patients or events to be powerful enough statistically.

The point of this review is to answer the following question: what are the negative and positive interactions between statins and omega-3 PUFAs in human? This leads, in case of positive interactions to the next question: does a co-prescription will improve the benefit-risk ratio compared to statins alone? We focused our investigation to the 3 main targets in cardiology: atherosclerosis, dyslipidemia and coronary heart diseases.

Discussion

I Interactions of Statins and ω3 PUFAs on Their Respective Metabolisms

In a RCT with 120 men having statin-treated hypercholesterolemia, there was a statistically significant decrease in ω3-PUFAs levels and an increase in ω6-PUFAs levels without an increase in total plasma levels of fatty acids (Anti *et al.*, 2005). The authors concluded that these results are due to a probable change in the chemical equilibrium of the PUFA metabolism enzymes (D5 and D6 desaturase) in favor of the formation of ω6 derivatives. Consistent with this result, there is a significant decrease in linoleic acid in the group under statins, evoking an acceleration of the transformation of this essential fatty acid in its derivatives ω6. Harris *et al.* (2004) sought to evaluate the specific effect of simvastatin on blood levels of ω3 and ω6 PUFAs in 106 healthy adults with hypercholesterolemia receiving either placebo or 40 mg simvastatin for 24 weeks [11]. Simvastatin reduced the concentration of all fatty acid classes and total blood concentrations of total FA. However, there are three notable exceptions: it did not affect arachidonic acid (AA), EPA and DHA levels. But it altered the relative blood levels of PUFAs in favor of ω6 which is deleterious to health.

The effects of statins on ω3-PUFAs varied depending on studies: some showed an increase, others showed no change. That is why Nozue *et al.* (2015) hypothesized that the effect of statins on the ω3/ω6 ratio could depend on the type/power of statin [12]. Therefore they tested the effect of 2.5 mg/d of rosuvastatin (a high-density, hydrophilic, older-generation statin) and 2 mg/d of pitavastatin (a high-density, lipophilic, a new generation statin) on PUFAs blood levels and their ratios in 46 patients with dyslipidemia. They found that in both groups DHA level as well as the DHA/AA ratio decreased but EPA/AA ratio did not change significantly. Considering the results of this study, it appears that the high-intensity statins as rosuvastatin, pitavastatin, and atorvastatin primarily affect DHA and DHA/AA. According to other studies, first-generation low-intensity statins (pravastatin and simvastatin) could primarily reduce the EPA/AA ratio. Nevertheless, it seems that it is not only the gross dietary intakes of PUFAs that are directly correlated with cardiovascular benefits but more specifically the circulating levels of EPA and DHA which are also secondary products of the endogenous metabolism of α-linolenic acid. In this line, in 2009, Lee *et al.* found that the plasmatic level of EPA, in 508 patients, is a predictive independent factor of mortality in the 16 months following a myocardial infarction [13].

To explain the negative effects of statins on ω3 PUFAs homeostasis, different explanation have been proposed. On one hand, Bird *et al.* evoke a physiopathological hypothesis that may explain this differential effect of ω3 PUFAs with or without statins [4]. They suggest that long-chain ω3 PUFAs found in the alimentation could induce a chronic state of preconditioning of the myocardium associated with an increase in ω3 PUFA accumulation in cardiac mitochondria. Indeed, myocardial mitochondria provide energy for ischemic preconditioning of cardiomyocytes before MI, which can reduce the extent of cell death and thereby decrease post-ischemic arrhythmias and improve patient survival. The endogenous production of ubiquinone (coenzyme Q10), which is used primarily to generate energy in mitochondria, is dose-dependently reduced by statin therapy because its biosynthesis requires the enzyme HMG-CoA reductase. Therefore, in the presence of statins, ω3 PUFAs may not be able to precondition cardiomyocytes due to a reduction of mitochondrial function resulting from an intrinsic ubiquinone deficiency.

On the other hand, statins promote ω6 PUFAs metabolism, which could result in ω3 PUFAs synthesis leading to a reduced ω3/ω6 ratio. Indeed, previous studies have indicated that statins may affect long-chain PUFAs

in terms of their synthesis and composition in tissues, in particular by increasing the concentration of AA. AA is converted by COX (cyclooxygenases) and LOX (lipoxygenases) into prothrombotic and proinflammatory eicosanoids, whereas EPA and DHA are anti-inflammatory and anti-thrombogenic. The focus is often put on the relationship between ω6 and ω3 PUFAs ratio because the production of pro-inflammatory and pro-thrombotic eicosanoids is facilitated by a preponderance of AA on ω3 PUFAs in the membrane pool of phospholipids. Thus, low basal serum ω3 levels may require more substantial supplementation.

In 2004, Harris & Von Schacky proposed the Omega-3 Index as a new risk factor for death from coronary heart disease (CHD) [14]. It would predict complications from CVD. The membrane composition of the red blood cells in FA reflects the ω3-PUFAs content of the cardiac membrane, namely the long-term intake of EPA and DHA, that is to say, the Omega-3 Index. They deduced that the Omega-3 Index is inversely associated with the risk of death from CHD. An omega-3 index >8% (*i.e.* high) allows for better cardioprotection and would result in a low risk of cardiovascular complications. On the other hand, an index <4% (*i.e.* low) leads to an increased risk of cardiovascular complications but this deficit is likely to be reduced by preventive ω3-PUFAs supplementation. All this is to be done with caution because even though the amount of ω3 consumed is known, each person is metabolically unique with idiosyncrasies for digestion, absorption, tissue distribution, and cellular metabolism. Individual variations of *in vivo* conversion of alpha-linolenic acid to EPA and DHA, as well as other dietary variables, can influence tissue levels of EPA and DHA. These factors combine to produce different levels of omega-3 among people who consume the same amount of EPA and DHA.

As a result, the Omega-3 Index can be useful to assess, first the baseline risk (especially for index <4%), secondly the variation in cardiovascular risk because the ω3 PUFAs absorption metabolism is specific to each individual [14]. To assess these findings, further randomized clinical trials are needed. Omega 3 PUFA can be found in the diet as in oily fish for example, but also as a dietary supplement. Currently, there are two main commercialized forms of omega-3 supplementation:

i. omega-3 acids ethyl esters (OM3EE) known as Lovaza® or Omacor®.
ii. icosapent-ethyl (IPE) known as Vascepa® (former AMR101®) which contains highly purified EPA ethyl ester.

The first form, available in the form of 1 g/d capsules, reduces TG, especially in patients with a rate > 5 g/d. However, an increase in LDL has been found, which runs counter to current recommendations. In contrast, IPE drastically reduces TG (33% for a 4 g/d dose) without increasing LDL (alone or in combination with a statin). Besides, a significant decrease has been demonstrated for non-HDL, VLDL and ApoB. It is, therefore, more appropriate, in the case of patients with dyslipidemia, to supplement them with this formula.

Note that a pharmaceutical form containing ω3 free fatty acids called Epanova® is under development. Numerous studies (EVOLVE or ESPRIT) have shown that this purified mixture of EPA and DHA forms has a greater effect on TG reduction [15]. Presently, omega-3 PUFAs could be useful in the treatment of major persistent hypertriglyceridemia events under statin and dietary hygienic rules [16]. In this study, EPA (IPE) is tested in 420 patients under statin, against statin and placebo. A blood lipid profile was performed before and after the intervention. While no modification of lipid parameters was observed in the control group, in the IPE group, a significant 21% decrease of TG was observed and a slight decrease of HDL, LDL, and ApoB. Also, a significant decrease in the size of lipoproteins was observed, especially VLDL and HDL. These results confirmed previous observation by the same group [17]. It is now accepted, and recognized by the National Lipid Association, that non-HDL cholesterol is a much better marker of cardiovascular risk because it takes into account all serum atherogenic particles. That is why the second target considered is non-HDL: a significant fraction of it is contained in the VLDLs. It therefore provides a more accurate index of atherogenic risk when TG are high (greater than 2 g/d). In patients with mixed dyslipidemia, a prescription of OM3EE is used as a complementary treatment for statins. It is an FDA approved supplement, for the treatment of very high triglyceride levels (above 5 g/d); and is available in 1 g capsules containing 465 mg EPA and 375 mg DHA.

Studies have been conducted on the pharmacokinetics of OM3EE when associated with statins. The results show that the level and extent of exposure (AUCt, Cmax, ss) to statins and its active metabolites were similar in the presence or absence of ω3 PUFAs. There was no pharmacokinetic impact in equilibrium conditions. The fact that statins depend on cytochrome P450 2C9 or 3A4 metabolism did not matter, whether observed in sick or healthy patients [18]. We can, therefore, say that the administration of OM3EE does not affect the bioavailability of statins and could be a therapeutic option in co-prescription with statins for patients with persistent hypertriglyceridemia [19]. Regarding the absorption of ω3-PUFAs, one of the first criteria to take into account is the specific molecular configuration that will occur in enterocytes. The ethyl ester forms require an additional digestion step with a bile salt-dependent carboxyl ester. In the case of high-fat meals, the bile will be at its maximum concentration. Therefore, the digestion and absorption of OM3EE appear to be highly dependent on the contents of the meal. This is not the case for the ω3 free fatty acids form, which is not dependent on the activity of the pancreatic enzymes.

Due to similar properties of statins and ω3 PUFAs on blood profile, Scolaro *et al.* emitted the hypothesis that an ω3 PUFAs rich diet could compensate for the diminution of statins doses to reach the same improvement of the blood lipid profile [20]. To challenge this hypothesis, they compared a group of patients with high doses of statins with another group having half the dose of statins and a diet made of fish oil, enriched in black chocolate (rich in vegetal sterols) and green tea (for polyphenols) in cross over in two periods of 6 weeks after a washout of 6 weeks. After supplementation, they observed a significant decrease of total cholesterol (-10%, p=0.002), LDL (-13%, p=0.002) and non-HDL cholesterol (-12%, p<0.001). The decrease of LDL was independent of lathosterol, a marker of sterol synthesis, but significantly dependent on campesterol, a marker of sterol absorption, suggesting that patients having a good "absorption" profile could respond better to nutritional supplementation. Interestingly, in a subgroup of patients having the most perturbed lipid profile, a 17% decrease of TG was observed. It can thus be proposed that nutritional supplementation could be complementary and/or an alternative to statins in patients resistant or inobservant. This could be an important improvement in personalized medicine. Moreover, ω3 PUFA could have a negative feedback effect on the activity of enterocytes transferases (inhibition of TG synthesis from FA) that could increase the degradation of ApoB48 just synthetized.

Compared to the literature, the new formulation of ω3 free fatty acids (Epanova®) has been developed to be taken during a low-fat diet. In a first clinical trial, ECLIPSE, a supplementation with free fatty acids forms of EPA and DHA has been compared to OM3EE. It has been shown that the free acid forms have a better bioavailability (4 times higher) than the current reference (omega-3 ethyl ester), either in terms of rate or extent of exposure. This offers a potential therapeutic benefit for the treatment of severe hypertriglyceridemia, as these patients follow a low-fat diet [21]. This study has been completed by the ECLIPSE2 clinical trial in which patients had to follow a diet low in fat and supplemented with vitamin K. The magnitude of difference in bioavailability was much greater in the area under the curve in patients receiving Epanova®. The serum TG level was significantly lower and the variability was reduced by a half. Therefore, it was concluded that the bioavailability of free forms of EPA and DHA for Epanova® had much better bioavailability than Lovaza® ethyl ester forms, under low-fat diet which are conditions normally recommended for patients having a CVD. This improvement could lead to improved TG reduction in patients with hypertriglyceridemia [22].

II Atherosclerosis

Atheromatous plaques are made of fibrinous elements that decrease the arterial compliance, proliferative smooth muscle cells that increase the vasoconstriction reflex and of lipidic elements, including the central necrosis, that, depending on the speed of its formation, is responsible for the atheromatous lesions instability [23-24]. The persistent inflammation inside the plaque increases local needs of nutriments and oxygen leading to a neo-vasa-vasorum formation that would, in turn, monopolize nutrients and oxygen toward the atheromatous plaque cells instead of those of the external healthy layers. Moreover, phospholipase A2 (PLA2) facilitates the oxidation of LDL particles. These oxidized particles then generate an inflammatory process of atherosclerosis. Finally, the proinflammatory mediators inhibit collagen synthesis, and the macrophages, that internalize cholesterol, produce matrix metalloproteinases (MMP) that weaken the fibrous shape leading thus to the plaque rupture and the thrombus formation.

Statins play a key role in atherosclerosis reduction. Indeed, the meta-analysis performed by Tian *et al.* showed that there is a significant reduction of the atheromatous plaque in patients taking at least 10 mg of statins since at least 6 months [25]. It has to be noticed that the reduction of the volume of the plaque was significant only for patients reaching the LDL target of < 1g/l. A reduction of LDL under 0.7 g/l is questionable since the reduction of the plaque volume is rather low (3.2%). However, Taguchi *et al*. showed that there are two populations of patients based on their sensitivity to statins: those for whom statins reduce the plaque volume and those for whom the plaque volume still increases after 8 months under statins [26]. Thus, statins reduce the number of cardiovascular events but still, residual risks persist such as atherosclerotic plaque progression. Other therapeutic options are therefore necessary such as addition of DHA and EPA to a statin to reduce the volume of a coronary atheromatous plaque, to prevent their progression. Indeed, higher blood levels of omega-3 fatty acids, EPA and DHA, have been associated with fewer cardiovascular events and lower mortality in prospective studies (as in the JELIS study, detailed below).

The question of the link between inflammation and atheromatous plaque has been reviewed in two animal studies. The first aimed to determine the biological role of pitavastatin + EPA combination on an animal model (rabbit). Yuki *et al*. have therefore damaged carotid rabbit who have undergone a 4 weeks-normal-diet, then the diet was enriched with cholesterol [27]. The authors found that pitavastatin and EPA combination inhibited the creation of atheromatous plaque by a decrease in the expression of inflammatory molecules such as MMP-9, tissue factor, and NF-kB. Nakajima *et al*. also showed in mice that EPA could be able to decrease the level of pro-inflammatory cytokines and chemokines (including interferon γ and TNFα) [28]. *In vitro*, it can regulate the phenotype of dendritic cells and the proliferation of T cells in lymphoid tissues, before their influx toward atheromatous plaques. Nishio *et al.* investigated the issue of plaque vulnerability, characterized in other studies by thin fibrous-cap thickness, large lipid pools, and macrophages infiltration of near the fibrous-cap [29]. They showed that in the rosuvastatin + EPA group (1.8 g/d), the rate of PTX3, reflecting the level of local arterial inflammation which plays an important role in the vulnerability of plaque, was lower in comparison with the statin group. Moreover, the fibrous cap thickness of the atheromatous plaque was significantly increased and macrophage accumulation decreased, as PTX3 level lowered. They concluded that in patients with untreated dyslipidemia, concomitant use of EPA and rosuvastatin may more effectively stabilize vulnerable plaques than statins alone.

Similarly, Urabe *et al.* using a cohort of 172 elderly people (mean age of 70 years old) having cardiovascular risks, showed that coronary atheromatous plaques stability is correlated to EPA and DHA plasmatic level [30]. A low rate of EPA is statistically associated with the presence of low-density plaques with an important remodeling, thus plaques with a high risk of rupture. Alfaddagh *et al.*, concluded from their study that a combination of high doses of EPA and DHA ethyl ester (3.36 g) with a low-intensity statin for 30 months provides additional benefits in preventing the progression of fibrous portion of the coronary plaque in observant subjects with a well-controlled LDLc level <0.8 g/l [31]. They raised the fact that subjects younger than 64.2 years of age had significantly less non-calcified plaque progression and less fibrous, calcified and total plaque volume compared to older subjects. This seems to suggest that more EPA and DHA are introduced early in the disease course, the more beneficial they may be.

They pursued their work in another study, focusing this time on the Omega-3 index. The same dose of EPA and DHA was used with the same duration of treatment [32]. They concluded that a dose of 3.36 g of omega-3 fatty acids added to statins over 30 months prevents progression of coronary artery plaque in non-diabetic subjects with LDL cholesterol and triglyceride (TG) well-controlled levels when the omega-3 fatty acid index above or equal to 4% is reached. Similarly, a low Omega-3 index, less than 3.43%, has been identified in non-diabetic subjects as being at risk for significant progression of coronary artery plaques despite statin therapy. The residual risk under statins could be explained by a low ω3/ω6 ratio, associated with cardiovascular events. Then, Nozue *et al.* sought to determine, through 2 post hoc analyzes of TRUTH, its effects on coronary atherosclerosis, especially in patients treated with different types of statins, namely pitavastatin (4 mg/day) and pravastatin (20 mg/day) [33]. It was concluded that a decrease in the ω3/ω6 ratio is associated with progression of coronary atherosclerosis during treatment with pravastatin but not with pitavastatin. Thus, patients with a decrease in the DHA/AA ratio and treated with pravastatin should be supplemented with ω3 PUFAs. This ratio, on the other hand, has less impact on coronary atherosclerosis in patients

treated with a strong statin due to a greater reduction of LDL (which is the most cholesterol-involved form of atherosclerosis).

In another study, Nozue *et al.* concluded that the decrease in the ω3/ω6 ratio is associated with progression of coronary atherosclerosis in statin-treated patients with coronary heart disease (CHD) [34]. Indeed, EPA and DHA levels at 8 months were significantly lower among subjects with atheroma progression. Thus, the variation of the (EPA+DHA)/AA ratio is a significant predictor of the percent change in plaque volume and volume of the fibrous component. This suggests that the decrease in the ω3/ω6 ratio is a residual risk of plaque progression in patients with CHD under statin therapy. Another study released in 2017 by Watanabe *et al.* followed a similar strategy but was designed to prevent coronary atherosclerosis by adding EPA therapy (1800 mg/d) for 6 to 8 months in 193 patients with coronary artery disease receiving high dose of pitavastatin (4 mg/d) [35]. This combination showed a significant reduction in coronary plaque volume compared with pitavastatin therapy alone, especially in patients with stable angina. The authors, therefore, concluded that the addition of EPA is a promising option to reduce the risk of coronary heart disease under intensive statin therapy.

However, it must be noticed that Jinhee *et al.* obtained opposite results [36]. In their study, they tested the effect of 3 g ω3-PUFAs. Coronary angiography did not show any difference neither in the rates of late loss, the difference between the minimum diameter of the post-procedure lumen and during the follow-up by coronary angiography, nor in the rate of in-stent restenosis.

Similarly, intravascular ultrasound did not show either a significant decrease in atheroma volume index in the ω3 group compared with the placebo group nor in the volume percentage of atheroma. They concluded that adding ω3 PUFAs to statin therapy may not have additional effects on the regression of coronary atherosclerosis in patients with coronary artery disease requiring a stent. However, it must be noted that in this single-blinded study they compared 2 groups who do not originate from the same patient population. On the other hand, the level of ω3-PUFAs (Omega 3 Index) was not determined and the authors did not control the consumption of fish that render difficult the analysis of the link between ω3-PUFAs and atherosclerosis.

III Dyslipidemia

High LDL level is a predictor of CVD risk; that is why it has been the target of treatment for dyslipidemia during the last 30 years. However, the residual risk persists despite LDL levels below 0.7 g/d, prompting a re-evaluation of the role of other lipoproteins as incremental risk predictors. The levels of TG and non-HDL appear to be additional risk factors, particularly in patients with mixed dyslipidemia, obesity, type 2 diabetes or metabolic syndrome. To date, there are several classes of drugs that lower TG, including fibrates, niacin and ω3-PUFAs. While the effects of fibrates and niacin were disappointing when combined with a statin, there is a significant reduction in the use of high-intensity statin-associated with ω3-PUFAs [37]. However, in this study, the authors did not indicate the EPA and DHA content, nor under which chemical structure they were synthesized. However, as seen above, the formulation will determine the pharmacokinetic properties of omega-3 and therefore their effects. This conclusion is, therefore, to be qualified. A prospective RCT was conducted to evaluate the safety and efficacy of the combination of statins and ω3-PUFAs in patients with dyslipidemia. One group received atorvastatin 20 mg and ω3-PUFAs supplementation while the other received only one treatment and one capsule of olive oil. A 30% reduction in triglyceride level and a 10% reduction in non-HDL were observed in the treated group. The addition of ω3-PUFAs could, therefore, be beneficial for patients with hypertriglyceridemia.

In 2013, the American College of Cardiology issued recommendations highlighting statins as a gold standard treatment. However, in light of new literature data, the Food and Drug Administration (FDA) recently approved the use of a new formulation containing ω3 fatty acids (containing 55% EPA and 20% DHA) as a supplement in diet to reduce TG in adults when it is ≥500 mg/dL. We will, therefore, discuss the effects of this new supplementation. Benes *et al.* wished to discuss the benefits of ω3 free fatty acids, both in monotherapy and in combination therapy with a statin, in reducing cardiovascular risk, particularly in patients with hypertriglyceridemia and low HDL cholesterol [38]. This new formula is based on the free form of fatty acids, which, as we have seen, is not impacted by the carboxyl ester lipase. It could, therefore, bring new results and new therapeutic leads. Subgroup analysis suggested a reduction in the number of major cardiovascular events with or without the concomitant use of statins. An increase in HDL was measured with doses of 2 or 4 g/d of omega-3 free fatty acids associated with a significant decrease in TG. Comparing with supplementation with DHA or EPA, the results are even more conclusive. Thus, additional treatment with ω3 free fatty acids could potentiate the benefits of statins, or provide benefits for those who are statin-intolerant or unwilling.

In the EVOLVE study conducted by Kastelein *et al.*, patients were counseled on therapeutic lifestyle changes (diet, sport, nutrition) [39]. One control group was supplemented with olive oil at 4 g/d, while three other groups received ω3 PUFAs in the form of free fatty acids at either 2 g/d, 3 g/d or 4 g/d. Bioavailability is five times higher in the treated patient groups, starting with supplementation of 2 g/d while TG were lower (20% on average). Finally, we can say, following the results of a study conducted by Maki *et al.*, that this new formula allows a reduction in the rate of non-HDL-C in patients [40]. Besides, total cholesterol and VLDL-C were found to be significantly reduced. Total cholesterol ratios on HDL-C and ApoA1 on ApoB were also significantly lower. Increased levels of plasmatic EPA and DHA were associated with a decrease in AA. It has to be noticed that EPA remains one of the reference supplements. The capacity of ω3-PUFAs to reduce triglycerides is admitted since a long time now [41, 42]. For example, Bays *et al.* showed, in 229 patients having persistent medium hypertriglyceridemia (between 0.5 and 2 g/l), that an extract containing 96% EPA (AMR101) reduces the plasmatic level of TG [43]. Compared to a placebo, 2 g/day of AMR101 during 12 weeks induced a significant decrease of 19.7% in circulating TG. This effect is dose-dependent : indeed taking 4g/day of AMR101 in similar conditions led to a decrease of 33.1% in TG. While the level of LDL is not modified, however it is of interest to notice that the HDL level is increased.

This effect could be consecutive to the negative regulation of genes involved in hepatic fatty acids synthesis and the increased expression of genes involved in the mitochondrial beta-oxidation. Both would lead to a lower biodisponibility of fatty acids to synthesize triglycerides. Such a decreased TG disponibility induces a decreased production and secretion of VLDL by the liver, which is one of the main lipoproteins carrying TG, with chylomicrons. Nevertheless, in this study, the occurrence of adverse events was identical in all three groups (AMR101 4g/d, AM101 2g/d and

placebo). They consisted of mild to moderate gastrointestinal disorders and gouty arthralgia, which led to discontinuation of the protocol in 4 patients, including 3 in the placebo arm. Also, there is no evidence of AMR101-induced biological changes in liver enzymes, CPKs, fasting glucose, or HbA1c. In short-term trials, supplementation with 4 g/day of EPA in patients with an average TG of 6.8 g/l resulted in a 33% reduction in this rate and a 22% reduction in patients treated with a statin. This was accompanied by a reduction of inflammation biomarkers (CRP, PLA2, ApoCIII) probably by a leukotriene-mediated effect [44].

To differentiate the effects of EPA and DHA intake, the MARINE trial investigates the efficacy and safety of AMR101 (IPE) in reducing TG and other lipid parameters in patients with very high TG (≥ 5 g/l). AMR101 is a formula consisting of ω3 fatty acid containing ≥ 96% EPA ethyl ester and no DHA [43]. Patients were supplemented with 2 g/d or 4 g/d. AMR101 4 g/d did not significantly increase LDL as did AMR101 2 g/d. But it significantly reduced non-HDL, apoB, PLA2, and VLDL. This could be of clinical significance because non-HDL represents all of the cholesterol carried by atherogenic lipoproteins (LDL, VLDL, IDL, chylomicrons, and lipoproteins). The decrease in TG was even better in patients receiving a statin, suggesting a possible synergy between AMR101 and statins. The authors concluded that non-HDL and ApoB may be better predictive factors for coronary heart disease than LDL. Reducing these lipid parameters with AMR101 may be clinically relevant in the management of patients with hypertriglyceridemia [43]. Finally, similar results were found in the ANCHOR study in patients with hypertriglyceridemia >2g/l but <5 g/l [16]. Patients with high TG levels in the serum have often elevations of non-HDL cholesterol. This type of cholesterol was identified as a second therapeutic target in these patients, but treatment goals were not achieved with a statin alone.

Therefore, to evaluate the efficacy and safety of a combination of dietary advice, simvastatin and the addition of a prescription OM3EE, a multicentric study in 41 centers was conducted on american soil [45]. The reduction of TG was sought, without, however, mitigating the fall in LDL. The patients received 40 mg/day of simvastatin and 4 g/d of OM3EE (Lovoza®) for the OM3EE + simvastatin group. OM3EE was associated with significant reductions in TG (30%), VLDL cholesterol, total cholesterol/HDL cholesterol, and a significant increase in HDL cholesterol, with all of these results $p < 0.001$ compared to the group control. The combination of OM3EE and simvastatin, therefore, seems to improve the entire lipid profile without decreasing the efficacy of statins. Besides, an examination of the changes in LDL particle concentration in the COMBOS study showed that total circulating concentration of LDL particles was not impaired by OM3EE treatment and that increase in LDL cholesterol was attributable to an evolution towards higher levels of cholesterol-rich LDL particles. Finally, a significant improvement in non-HDL cholesterol levels with the decrease in TG due to the addition of OM3EE to simvastatin was found.

In a posthoc analysis of data from the COMBOS study, authors have therefore sought to determine changes in LDL levels in response to the prescription of OM3EE therapy in women and men with high TG (200 to 499 mg/dL) associated with diet and statin therapy [46]. The reduction in VLDL concentration was greater than the increase in LDL, and a clear decrease in cholesterol concentrations carried by atherogenic particles (non-HDL cholesterol) was observed. These results suggest that the increase in LDL that occurred with the addition of OM3EE to simvastatin treatment among mixed dyslipidemia patients was mainly reduced among low LDL patients receiving simvastatin monotherapy. In a desire to evaluate the efficacy and safety of statin and Omacor® (850mg EPA plus DHA) combination, a study conducted in South Korea, in 33 health centers, showed that in the supplemented group, the decrease in TG and non-HDL was significantly higher than in the gold standard group. Total cholesterol, ApoA1, ApoB, and VLDL were also lowered [47]. Regarding side effects and occurrence of adverse events, there was no reported increase in the supplemented group compared with the control group.

IV Coronary Heart Disease

The positive effects of statins on cardiovascular mortality have been well documented. For example, a meta-analysis of 27 RCT including 174000 patients followed during 5 years in average shows that statins are responsible for a significant decrease of 24% in major coronary events as well as coronary revascularization procedures and a decrease of 12% per 0.4 g/l of decrease LDL in cardiovascular mortality [5]. It must be noticed that the authors underline a persistent cardiovascular risk for a level of LDL above 1 g/l. This can be explained by the inability of some patients to reach the objectives presently recommended, even if they have good observance and maximal doses traitement. The variability of patients to reach LDL target has been observed in some studies. By studying the prevalence of residual dyslipidemia under statins in a multicentric transversal observational cohort of 4282 patients having an anomaly of the lipid profile in the last six months, Gitt *et al.* observed that, even with a good observance, only 20% of patients have a normal lipid profile, 25% have an abnormal LDL level, 33% have at least one of these two anomalies (HDL or triglycerides) even if the LDL is as expected [48]. In general, 67% do not reach the expected total cholesterol and 60% do not reach the targeted LDL. Even in patients reaching the targeted LDL, there are still cardiovascular events. The level of TG appears as a residual marker in the appearance of its events. Moreover, previous analyses have determined the extent to which icosapent-ethyl (AMR101®) reduced the number of total ischemic events. It has also been reported to have anti-inflammatory properties and atheromatous plaque stabilizers.

Though, most of the recent clinical trials have found no benefit [7]. It should be noted, however, that for several years, most of the patients included in secondary prevention studies have been treated with statins. It is therefore likely that there might be interactions between ω3 fatty acids and statins. A cohort comparing statin users and non-users concluded that the risk of CVD mortality is decreasing as the ω3 PUFAs consumption increases, but only in non-statin users group [49]. However, it is important to note that, despite considerable advances in statins, ischemic events continue to occur in patients with cardiovascular risk factors, such as high TG, atherosclerosis, or diabetes. Besides, there is a risk of recurrence after the initial event that could be fatal. The residual risk of cardiovascular events that persist after high-intensity statin therapy may be explained by low levels of ω3 PUFA, particularly EPA and DHA. Kurisu *et al.* showed that high-intensity statin hypolipidemic therapy mainly reduced ω3 PUFAs (dihomo-γ-linolenic acid, EPA and DHA) in proportion to the decrease in LDL cholesterol levels in patients with CHD [44]. Complementary treatment with ω3 PUFAs may be one of the solution to resolve the residual risk in patients with CHD who are treated with a statin alone, because of the evidence that low ω3 PUFA is a risk factor for cardiovascular events.

To understand better a possible role of ω3 PUFAs, it is not enough to take into account only the daily intake of these PUFAs (through the diet and/or enriched-pills) due to the large variability of incorporation [50]. The majority of RCT in the early 2000s demonstrated a beneficial effect of omega-3 fatty acids on health, especially in the secondary prevention of cardiovascular disease. In 2002, based on the results of the GISSI-Prevenzione trial, the AHA recommends the consumption of two meals of fatty fish per week in the general population and supplementation of 1 g/day of EPA and DHA if subjects have coronary heart disease. The JELIS trial is one of the studies with the most perspective on the contribution of EPA [51]. Conducted over an average of more than 4 years in subjects all treated with statins, it has been shown a 19% reduction in coronary clinical events (angina, coronary events) among patients supplemented with EPA. In the case of people who already had an MI or had CHD, supplementation reduced the recurrence risk by 19%, including a 28% decrease in angina. A decrease in TG and a 25% reduction in LDL cholesterol were observed in both groups. Authors concluded that supplementation with EPA prevents the occurrence of coronary clinical events in Japanese patients with hypercholesterolemia. Nevertheless, it is important to note that this article was based exclusively on the Japanese population and subjects educated to eat mainly fish as a source of animal protein.

Macchia *et al.* investigated the prevalence of fatal or non-fatal cardiovascular events in patients discharged for MI and found that between 2 and 3 events per 100 patients per year could be avoided in the statin + ω3-PUFAs group counter to the statin group alone [52]. However, their study had several flaws, the main is the design (retrospective cohort), which did not provide information on patients' cardiac function (left ventricular ejection fraction).

In the REDUCE-IT study, it was hypothesized that the risk of CV events would be lower with high-dose IPE (4 g/d) than with placebo, among patients who have a high level of TG, despite statin therapy [53]. This trial was conducted in 11 countries and more than 470 sites. A significant reduction of major CV events was found in the IPE group (25%), regardless of the TG level achieved at 1 year (≥ 150 or <150 mg/dL) suggesting that CV risk reduction was not associated with achieving normal TG. These observations suggest that, at least, some of the effects of IPE that resulted in a lower risk of ischemic events compared to placebo, may be due to metabolic effects other than lowering TG levels. In conclusion, among patients with high levels of TG and treated with a statin, the risk of major ischemic events (including CV cause death) was significantly lower (4.8%) among those who received 4 g/d of IPE.

Following this release, the authors focused on the effects of IPE on total ischemic events to better characterize all ischemic events in the entire study population [54]. For every 1,000 subjects, 159 events could be avoided, giving a relative risk reduction of 30%. There was a slight significant increase in hospitalization observed for atrial fibrillation or flutter in the IPE group. But, the large number of reduced ischemic events with this drug (28% fatal stroke, 48% cardiac arrest, 31% sudden death and 20% CV death) indicate that the risk-benefit ratio is largely in favor of the benefit. Since the inclusion criteria were rather general and there were few exclusion criteria, these results are rather generalizable to a large population: that is, high-risk CV patients treated with statins and with atherosclerosis or diabetes. Therefore, among statin-treated patients with elevated TG and CVD or diabetes, many statistical models demonstrate that icosapent-ethyl 4 g/d significantly reduces first ischemic events, subsequent ischemic events, and total ischemic events. In these patients, IPE is an important treatment option for reducing the consequences of atherosclerosis-related events beyond statin therapy alone. EPA, therefore, appears to be a major therapeutic option in the treatment of hypertriglyceridemia and the prevention of CVD.

Three studies were placed in a particular frame namely the percutaneous coronary intervention (PCI). Nosaka *et al.* evaluated the effect of early initiation of a combination of EPA and statin therapy after percutaneous coronary intervention on reducing the rate of cardiovascular events after acute coronary syndrome (ACS) compared with statin monotherapy [55]. The authors concluded that treatment with 1800 mg/day EPA added to 2 mg pitavastatin is a promising therapy if it is started within 24 hours after percutaneous coronary intervention in patients with ACS, reducing the number of cardiovascular events and the death by cardiovascular cause. The study conducted by Yagi *et al.* focuses on the effects of statins on serum PUFA level and the subsequent impact on in-stent restenosis in patients with ACS, with the idea that lowering the blood level of ω3-PUFAs (in this case DHA) could be used as a target for prevention of in-stent restenosis after statin therapy [56]. The authors obtained, with the introduction of a statin in these patients, a decrease in blood levels of DHA in a dose-dependent manner as well as of LDL. Thus, the decrease in the DHA rate after statin therapy and a low level of EPA on admission are risk factors for in-stent restenosis.

Kurita *et al.* focused on the incidence of peri-interventional myocardial infarction [57]. Following a PCI, myocardial infarction can occur due to vasospasm in reaction to the catheterism. This kind of infarction is rather frequent and occurs mainly in patients already suffering from coronary disease. This study has been conducted in 165 patients following a PCI after treatment with statins alone or supplemented with 1.8 g/d EPA. The outcome was an elevation of the level of troponin above 0.1 ng/mL, this outcome being admitted as a diagnostic criterion of myocardial infarction. In the group under statins alone, 45% of the patients had an elevation of troponins for which 6% the elevation was above 0.5 ng/mL. In the group statins plus EPA, the troponin elevation was just 14% for which 1% of the patients presented a troponin elevation above 0.5 ng/mL. Interestingly, the authors observed a negative correlation between variations of troponins and the plasmatic level of EPA.

Conclusion

Ω3 PUFAs have been recognized as being cardioprotective until the mid-2000's as well as the systematic prescription of statins after myocardial infarction. Even if a recent analysis of the litterature led to advise ω3 PUFAs in the secondary prevention of myocardial infarction but not to other cardiac diseases, the interest of ω3 PUFAs remains [6]. Differents studies showed that statins interfere with ω3 PUFAs homeostasis leading to a decrease in circulating EPA and DHA. In the same time, it has been shown that the Omega3 Index is a good predictor of the evolution of cardiovascular diseases, a low index being of bad pronostic.

To explain some discrepencies between clinical trials showing a beneficial effects of adding ω3 PUFAs to statin prescription, different hypothesis can be emitted. On one hand, the last clinical trials of supplementation having positive results used dose above 500mg/day but high-doses (≥2g/d) are generally used in statins-treated patients to observe a reduction of the atheromatic plaque volume, non-HDL cholesterol, … This need of using high-doses of ω3 PUFAs might be

explained by the fact that statins interfere with ω3 PUFAs homeostasis and it might be expected that at these concentrations, ω3 PUFAs have not their main side effect, haemorrhagic properties, since the concept is to normalize the circulating level of EPA en DHA [3]. On the other hand, ω3 PUFAs are generally used as one molecule while recent studies, REDUCE-IT for example, tend to prove that EPA and DHA have different properties, EPA being more efficient in curing dyslipidemia. ω3-PUFAs, and more specifically EPA, reduce TG levels and appear to have pleiotropic effects in reducing the instability of atheromatous plaque and mediators of inflammation. At the molecular level, EPA compared to DHA appears with more favorable effects on artery inflammation. Ω3 PUFAs could be used in personalized medicine in association with the determination of the Omega3 Index in order to control the circulating levels of EPA and DHA.

Conversely, it appears that DHA could have a more pronounced effect than EPA on the decrease of TG blood level and the increase of HDL (especially the fraction 2 of HDL that is thought to have the cardioprotective effects) and LDL (especially though its size). These differential effects could involve the modulation of transcription of apolipoprotein lipases. If we focus on coronary plaques, a low level of EPA is statistically associated with the presence of low-density coronary plaques with remodeling which are at high risk of disruption [30]. This link does not exist with DHA. On the opposite, the incidence of atrial fibrillation is reduced after supplementation with DHA (-6% per 1% increase in DHA) [58]. EPA has not such an effect.

To conclude, the use of ω3 PUFAs as an adjuvant therapy to prevent CVD in association with statins appears to present some advantages (lowering the dose of statins, reducing the markers of CVD and morlatity, …) that needs to be studied more accurately to be proposed to the benefit of the patients.

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