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Editorial

Dear Colleague,

In this first volume of the 2009 Lipid Club letter quarterly You will find three important contributions:

- a report with results from basic science focusing on the understanding of the molecular mechanisms involved in the activation of cultured human endothelial cells EAhY926 by LPA.
- a report summarizing results from the JUPITER trial, a RCT of rosuvastatin in a particular group of subjects free of CVD. This trial was ended prematurely; results were presented at the AHA meeting in 2008 and published in the NEJM.
- a report dealing with recommendations on the use of omega-3 fatty acids in the prevention of CVD.

These three reports clearly illustrate the diversity of what we are dealing with in the Belgian Lipid Club; they also emphasize the need for a continuous confrontation between observations in the laboratory and those made in the clinic and in the community.

Progress in the prevention, diagnosis and treatment of atherosclerosis and its clinical consequences can only be expected through joint efforts between multidisciplinary research teams that look at similar problems from different angles.

I wish You all a pleasant reading; I thank the authors and I look forward to meet You all at the forthcoming meeting of the Belgian Lipid Club.

Cordially

Prof. Dr. G. De Backer
President BLC.

AstraZeneca
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Pfizer
Solvay Pharma
Sankyo
Schering Plough

Responsible editor: Prof. G. DE BACKER (R.U. Gent) • Editorial staff: Prof. J. DUCOBU (ULB, UMH)
Atherosclerosis is an arterial degenerative disease characterized by a progressive obstruction of blood vessels, leading in its most aggressive form to myocardial infarction or stroke. Among the various cell types involved in atherogenesis, monocytes/macrophages and endothelial cells play a major role. Several factors may alter the integrity of the endothelium and among them bioactive lipids resulting from LDL (low density lipoprotein) oxidation, such as lysophosphatidic acid (LPA), have been recently the focus of increasing interest. There is a lot of evidence suggesting the involvement of LPA in the development of atherosclerosis, but other data rather favour a protective role of LPA, especially regarding endothelial cells. The aim of this study was to better understand the molecular mechanisms involved in the activation of cultured human endothelial cells EAhy926 by LPA. We first demonstrated that LPA induced IL-8 and MCP-1 overexpression, favouring monocyte THP-1 transmigration in vitro. We also observed that LPA was able to directly modulate THP-1 transmigration, in a concentration dependent way. We next investigated the effects of LPA on the proteins secreted by EAhy926 cells, using the 2D-DIGE approach to analyze the secretome. One protein showing significant increased abundance was identified by mass spectrometry and focused our attention: pentraxin-3, a member of the acute-phase protein family, including CRP/SAP. For the first time, we highlighted the chemotactic activity of pentraxin-3 towards THP-1 monocyte transmigration.

Cardiovascular diseases (CVD), such as atherosclerosis, are the most common causes of death in developed countries. Atherosclerosis previously considered as a passive process resulting from the accumulation of lipids in the artery wall with narrowing of the lumen and consequent myocardial ischemia, is now regarded as a dynamic process that involves inflammation at all stages, from the early to the complex lesions.1 Inflammation is enhanced by all the CVD risk factors identified in epidemiologic studies, and particularly by elevated levels of low-density lipoprotein (LDL) associated cholesterol.2 Oxidation of LDL is generally considered as one of the key events in atherogenesis and oxidized LDL (oxLDL) is a major source of various bioactive modified (lyso)phospholipids. Among those oxLDL-derived lipids, lysophosphatidic acid (LPA) has been identified and largely studied in order to elucidate its role in atherogenesis.3 LPA is a normal constituent of serum and plasma, present in the range of 80 nM to 0.7 µM in plasma and of 2-20 µM in serum, with palmitoyl- and oleoyl-LPA being predominant.4 LPA was primarily described as a growth factor, but it can provoke a large variety of different biological responses in many...
cell types (for a review see\(^1\)). LPA exerts its major biological effects by binding to specific transmembrane G-protein-coupled LPA\(_1\) receptors, but LPA also binds with high affinity to the nuclear receptor peroxisome proliferator-activator receptor \(\gamma\) (PPAR\(\gamma\))\(^2,7\). Vascular wall and blood cells express several types of LPA receptors and there is accumulating data indicating that LPA is a potentially athero- and thrombogenic molecule. Since it stimulates platelet aggregation, promotes proliferation of vascular smooth muscle cells (VSMC) and activates monocytes, macrophages, as well as vascular endothelial cells (EC)\(^8\). Activated EC, well known to interact with other cells such as monocytes, macrophages or platelets, play an important role in the context of atherogenesis by secreting proteins, such as pro-inflammatory interleukins (IL-1, IL-6, ...) and chemokines (monocyte chemotactic protein-1, IL-8, ...). We therefore investigated the effects of LPA on the proteins secreted by EC. First, we used the 2D-DIGE approach to analyze the secretome of LPA-treated EAhY926 cells, that allowed us to demonstrate a LPA induced pentraxin-3 overexpression. Secondly, we showed that LPA also induced IL-8 and MCP-1 secretion, two well-known chemokines implicated in the early stages of atherogenesis. Finally, as in vivo, the evolution of the initial atherosclerotic lesion relies on a complex interplay between in particular the endothelial cells acting as a secretory tissue and monocytes responding to them by transmigration, we demonstrated the chemotactic activity of these proteins using an in vitro chemotaxis assay.

Materials and Methods

Cell culture and LPA stimulation. The EAhY926 cell line is a hybridoma produced from human umbilical vein endothelial cells (HUVEC) fused with cells of the carcinoma A549 cell line (generous gift from Dr Cora-Jean Edgell, University of North Carolina, USA) and the leukemia THP-1 cell line was purchased from the ATCC. Cells were cultured as described in details by Gustin et al.\(^9\).

RNA extraction and cDNA synthesis. Real-Time reverse transcriptase-polymerase chain-reaction (RT-PCR) was used to quantify pentraxin-3 and GAPDH (chosen as housekeeping gene) transcript abundance. Briefly, RNA was extracted with the “RNAgent total RNA isolation system” kit (Promega), according to the manufacturer’s instructions. Real-time RT-PCR was performed using the SYBR Green PCR Master Mix.

ELISA. EAhY926 cells were seeded at 180,000/well in 24-well plates the day before stimulation. Pentraxin-3 and IL-8/MCP-1 released into the culture medium were measured using an ELISA kit respectively from Alexis and from R&D, following the manufacturers instructions.

Gene silencing experiments. Transfection experiments with small interfering RNA (siRNA) against pentraxin-3 were performed using a smart-pool of four specific siRNA (Thermo Fisher Scientific) targeting human pentraxin-3 (NM_002852). A control siRNA “RISC-free”, chemically modified to impair its uptake by RISC (RNA-induced silencing complex), was used as

Results

LPA induces IL-8, MCP-1 and Pentraxin-3 secretion in human endothelial cells

EAhY926 cells were treated with 25 µM LPA during 4 hours and the secretome of the LPA-treated cells was compared with untreated cells, using the 2D-DIGE approach and analyzed with the DeCyder software. Among the 20 spots displaying significant variations in abundance, 2 were identified by mass spectrometry as pentraxin-3.\(^9\) Both spots showed a significant increase of abundance in LPA-treated cells (P<0,0076 and P<0,0032, respectively) with a 1,54- and 1,51-fold induction, respectively. To confirm these data, we measured pentraxin-3 released into the supernatant of EAhY926 cells after 4, 6 and 8 hours stimulation with LPA at 25 µM (Figure 1). As measured by ELISA, LPA induces a pentraxin-3 release with a maximal effect between 4 and 6 hours. We next investigated whether LPA was able to modulate protein secretion of MCP-1 and IL-8. As observed in Figure 1, we showed that LPA (25 µM) upregulates IL-8 and MCP-1 secretion with a maximal effect after 4 hours,

Figure 2.
that declines thereafter. This upregulation of pentraxin-3, MCP-1 and IL-8 was also confirmed at the mRNA level by real-time RT-PCR, both in EAhy926 and in primary HUVEC cells 6, 9.

**LPA is one of the major bioactive phospholipids in oxLDL inducing pentraxin-3 overexpression**

Because LPA accumulates during LDL oxidation, EAhy926 cells were treated for 2 hours with 200 µg/ml of oxLDL and pentraxin-3 gene expression was monitored by real-time RT-PCR. oxLDL induced an increase of pentraxin-3 expression with approximately a 2.5-fold induction (Figure 2), almost comparable to the one observed in the presence of 25 µM LPA (Figure 1). Moreover, a preincubation with 10 µM Ki16425, an antagonist of LPA, and LPA, receptors, before the incubation with oxLDL, markedly diminished this overexpression to levels comparable to the negative control (Figure 2). Native LDL (LDL) and Ki16425 (Ki) alone had no effect. To check whether LPA was the major bioactive lipid responsible for this response, we tested 2 other lipids present within oxLDL, sphingosin-1-phosphate (S1P) and lysophosphatidylcholine (LPC), but no overexpression was observed (Figure 2). All together, these data demonstrate that oxLDL induces pentraxin-3 expression through LPA, and LPA₁ receptors in EAhy926 cells.

**LPA induces monocyte chemotaxis**

We first studied the capacity of LPA, over a large concentration range (from 100 pM to 100 µM) to favour or not THP-1 migration, using a modified Boyden’s chamber. By adding LPA to the lower chamber during 4 hours, we observed a typical bell-shaped dose-response curve since LPA enhanced migration from 100 pM to 100 nM with a maximum effect observed at 10 nM, whereas inhibiting this transmigration from 100 to 100 µM (Figure 3A). No effect was observed at 1 µM LPA. Next, to exclude any chemokinesis (random migration), we repeated the experiments by adding equal LPA concentrations in both the lower and upper chambers. In these conditions, LPA was unable to stimulate monocyte migration 9.

**Pentraxin-3, MCP-1 and IL-8 induce the transmigration of monocytes THP-1 in vitro**

Since LPA at 1 µM had no effect on monocyte migration, we tested conditioned media from EAhy926 cells treated with 1 µM LPA for 4 hours on monocyte transmigration. Since these conditioned media contain MCP-1 and IL-8, neutralizing antibodies against MCP-1 and IL-8 were added, combined or not, to the conditioned media of LPA-treated EAhy926 cells (4 hours at 1 µM). As shown in Figure 3B, these antibodies abrogate the chemotactic activity of the endothelial cell supernatants, but only partially. These results suggest that the LPA enhancement of the chemotactic activity in the supernatant of endothelial cells is largely mediated through secreted IL-8 and MCP-1. Because the functions of pentraxin-3 are not yet completely defined, we wondered whether pentraxin-3 could be also chemotactic for monocytes, favouring their transmigration. To test this hypothesis, pentraxin-3 expression was abrogated by using specific double-stranded siRNA. Conditioned media of EAhy926 cells stimulated with 1 µM LPA (for 4 hours) and transfected or not for 24 hours with siRNA against pentraxin-3 were tested on the THP-1 transmigration and displayed a significantly lower chemotactic activity (Figure 3B). Moreover, this inhibition was counteracted when 300 ng/ml of exogenous rhPentraxin3 (concentration achieved in the supernatant of endothelial cells after LPA treatment) were added during the transmigration assay. These data point out for the first time a new function of pentraxin-3 as a chemoattractant for monocytes.

**Discussion**

Endothelial cells respond to local injury by overexpressing adhesion molecules and actively secreting pro-inflammatory cytokines and chemokines, leading to monocyte chemotaxis followed by their transmigration through the endothelial barrier. In order to unravel some of the effects of LPA at the molecular level, we wanted to investigate its effects on gene expression focusing our attention on the secreted proteins. Recruitment of circulating monocytes into the subendothelial space, due to secretion of chemotactant factors such as IL-8 and MCP-1 by EC, is one of the early steps in lesion progression 10.

Hence, we first wondered whether LPA could stimulate endothelial EAhy926 cells to express these two chemokines. As already demonstrated by Lin et al. 11 on primary HUVEC cells, we showed that LPA induces MCP-1 and IL-8 at the mRNA and protein levels in EAhy926 cells, a model more and more characterized in several in vitro studies related to the context of cardiovascular diseases 12, 13.

We also observed similar results on primary HUVEC cells 6, 9. Secondly, we demonstrated, by a subproteome approach, that LPA clearly modulates the secretome of EAhy926 cells and significantly increases the abundance of pentraxin-3 9. Pentraxin-3, produced by a variety of tissues and cells, is the prototypic protein of the long pentraxins, one of the 2 subfamilies that compose the pentraxins. CRP and SAP belong to the group of classical short pentraxins and are produced by liver cells, more prominently in response to IL-6. The 2D-gel data on pentraxin-3 were confirmed by ELISA. Moreover, we demonstrated that oxLDL by themselves enhance pentraxin-3 expression through the activation of LPA₁ and/or LPA₃ receptors, suggesting the involvement of LPA in this induction. In order to further study the specificity of the LPA-induced pentraxin-3 in EC, we also tested other bioactive lipids present within oxLDL, such as S1P and LPC, but they were unable to modulate pentraxin-3 gene expression.

We next investigated the effects of LPA, as well as of conditioned media obtained from LPA-treated EAhy926 cells, on monocyte migration. First, our data clearly showed that LPA directly modulates monocyte chemotaxis, exerting opposing effects according to its concentration: monocyte migration is enhanced in the presence of LPA 100 pM to 100 nM with a maximum effect at 10 nM, while it is inhibited at higher concentrations (10 µM to 100 µM). The bell-shaped response curve obtained for monocyte chemotaxis in response to LPA is in agreement with the recent data obtained on breast cancer cells 14. Then, we wanted to challenge the monocytes with endothelial...
cells in the presence of LPA, as it occurs in vivo. Therefore, we demonstrated that conditioned media of 1 μM LPA-treated cells significantly enhanced monocyte migration, mainly because of the enhancement of IL-8 and MCP-1 secretion.

Finally, we investigated what could be the patho-physiological significance of pentraxin-3 secretion by EC. Previous studies have suggested a role for pentraxin-3 in the local control of the inflammatory process, in cytokine expression and, like for CRP, in modulating complement activity by binding to the complement component Clq. Recent emerging data suggests that CRP may play a direct role in the progression of atherosclerosis, by favouring the monocyte chemotactic response to MCP-1. We thus wanted to look for a possible chemotactic activity of pentraxin-3 on monocytes, an activity not assigned up to now to our knowledge to pentraxin-3. Pentraxin-3 gene expression was abrogated by using siRNA that indeed did reduce the chemotactic activity of the supernatants of 1 µM LPA-treated cells, but this activity was demonstrated with 300 ng/ml of human recombinant pentraxin-3. Pentraxin-3 has a clear chemotactic activity on human THP-1 monocytes, which is in favour of a proatherogenic role for pentraxin-3. These results are in agreement with some recent observations. First, pentraxin-3 is strongly expressed in atherosclerotic lesions compared to normal arteries. It has also been reported that pentraxin-3 is induced by oxLDL in VSMC. Latini et al have demonstrated the higher prognostic value of pentraxin-3 in acute myocardial infarction compared to the best-known relevant biological markers. Finally, very recently, Suzuki et al demonstrated that plasma pentraxin-3 levels were associated with the highest risk of cardiac events, suggesting that pentraxin-3 may constitute a new promising predictor for cardiovascular events.

In this context, our results suggest that pentraxin-3 and LPA may be new relevant therapeutic targets to consider for the treatment of inflammatory vascular lesions.

References


Figure 3. Effects of LPA and endothelial cell-conditioned media on monocyte migration. (A) Concentration-dependent effect of LPA on monocyte migration in an in vitro chemotaxis assay. Different concentrations of LPA diluted in DHG + 0.1% fatty acid-free BSA were tested on the migration of THP-1 for 4 hours. Results are given as means ± SD (n = 3). (B) Effect of endothelial cell-conditioned media on the migration of THP-1. EAhy926 cells were treated or not for 4 hours with 1 μM LPA and the corresponding conditioned media were tested on the migration of THP-1 monocytes, in the presence or not of anti-pentraxin-3, control siRNA, or specific siRNA that indeed did reduce the chemotactic activity of the conditioned media on the migration of THP-1 during 4 hours. Results are given as means ± SD (n = 3) and data are expressed as a percentage of control (Ctl = DHG + 0.1% fatty acid-free BSA). ***P<0.001; **P<0.01; *P<0.05 vs. Ctl.

In Figure 3b, the y-axis represents the migration of THP-1 cells (% of control) and the x-axis represents the concentration of LPA (−logM).

Figure 3a. Figure 3b.
The JUPITER trial (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) started the American Heart Association (AHA) 2008 Scientific Sessions. The field of primary prevention was shaken up by the results showing that the treatment of apparently healthy patients with a statin cuts their risk of cardiovascular disease morbidity and mortality by almost half. JUPITER is a large, multinational, long-term, double-blind, placebo-controlled, randomized clinical trial that included 17,802 healthy men and women with normal LDL cholesterol but elevated CRP (>2.0 mg/L) assigned to rosuvastatin 20 mg or placebo. (figure 1)

Among patients treated with rosuvastatin, LDL-cholesterol levels were lowered from a median 108 mg/dL at baseline to 55 mg/dL at 12 months. CRP levels were also significantly reduced, declining from 4.2 mg/L at baseline to 2.2 mg/L at 12 months. Triglyceride levels were reduced 17% from baseline among those treated with statin therapy. HDL was not changed. These effects persisted over the course of the study. (table 1)

Stopped after 1.9 years of follow-up, treatment with rosuvastatin significantly reduced the primary composite end point.

### Table 1: Baseline and change in LDL cholesterol and CRP levels during study period

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>12 mo</th>
<th>24 mo</th>
<th>36 mo</th>
<th>48 mo</th>
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<tr>
<td><strong>LDL cholesterol (mg/dL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosuvastatin 20 mg</td>
<td>108</td>
<td>55</td>
<td>54</td>
<td>53</td>
<td>55</td>
</tr>
<tr>
<td>Placebo</td>
<td>108</td>
<td>110</td>
<td>108</td>
<td>106</td>
<td>109</td>
</tr>
<tr>
<td><strong>High-sensitivity CRP (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosuvastatin 20 mg</td>
<td>4.2</td>
<td>2.2</td>
<td>2.2</td>
<td>2.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>4.3</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.3</td>
</tr>
</tbody>
</table>
44% compared with placebo. This reduction was observed among nearly all of the individual end points, including a 55% reduction in nonfatal MI, a 48% reduction in the risk of nonfatal stroke, and a 47% reduction in the risk of MI, stroke, and death from cardiovascular causes. (figure 2)

In terms of absolute benefits, the proportion of patients who had an MI, stroke, revascularization, hospitalization for unstable angina, or died from cardiovascular causes was 1.6% in the rosuvastatin arm and 2.8% in the placebo arm, an absolute risk reduction of 1.2%. Similarly, the proportion of patients with hard cardiac events — cardiovascular death, MI, and stroke — was reduced from 1.8% in the placebo arm to 0.9% in the rosuvastatin arm, an absolute reduction of 0.9%.

The all cause mortality (secondary endpoint) was decreased by 20% (p=0.02) (figure 3)

A subgroup analysis revealed no heterogeneity in any of the results, including an analysis of subgroups based on age, race, or ethnic group, as well as baseline LDL-cholesterol and CRP levels. The investigators report that even patients considered to be at very low risk — those who did not smoke, were not overweight, did not have metabolic syndrome, or had a Framingham risk score of 10% or less—benefited from statin therapy. (figures 4 and 5)

In terms of side effects, significantly more patients in the rosuvastatin arm developed new diabetes, but any reported serious adverse events were similar between the placebo and statin-therapy arms.

**Commentaries**

This results of this study are very important in the primary prevention area but, as usual, the absolute risk differences are less impressive than the relative differences and, together with cost and long-term safety considerations, will serve to temper the conclusions of the study.

In his NEJM editorial, Hlatky calculated that 120 patients need to be treated for 1.9 years to prevent one death from cardiovascular causes, MI, or stroke, and this benefit needs to be balanced against concerns about significantly higher glycated hemoglobin levels and increased diabetes incidence observed in the rosuvastatin arm. The JUPITER investigators, on the other hand, calculated the number needed to treat (NNT) based on the primary-end-point event and report that the NNT with rosuvastatin for two years to prevent one primary end point is 95 and just 31 need to be treated for four years to prevent one primary-end-point event.

Many patients with heart attacks have normal LDL-cholesterol values. Are they healthy? Doctors and patients are into a false sense of security when their LDL cholesterol is ‘normal’ and then are surprised when they have a heart attack. JUPITER illustrates this point perfectly. But if you look at the median values of Jupiter patients — age 66 years, body-mass index 28.3 kg/m², systolic blood pressure 134 mm Hg, and 41% with metabolic syndrome — you know that these people are going to have heart attacks and strokes. Indeed, in the placebo arm, the event rate was 1.36% per year.

Reducing LDL cholesterol while at the same time treating inflammation is one of the reasons why this treatment was so
successful. Two other studies, analyses of the PROVE-IT and REVERSAL trials, by Ridker and Nissen, respectively, previously showed that lower CRP levels are associated with fewer cardiovascular events independent of LDL-cholesterol levels. But JUPITER trial was not designed to explain if the observed results are due to the LDL lowering or to the decrease of CRP.

An important information is lacking until now in the JUPITER results: it would be interesting to know the variations of the CV events, according to the tertiles of CRP decreases…

**Should every patient undergo CRP testing?**

After JUPITER, guidelines are likely to be revisited, although it is cautious not to predict the changes of the findings will have on clinical practice.

JUPITER provides more evidence about the effectiveness of statin therapy in reducing cardiovascular risk, even among persons who would not currently be considered for pharmacotherapy. Guidelines for primary prevention will surely be reassessed on the basis of the JUPITER results, but it depends on the balance between the benefits of treatment and its long-term safety and cost.

In his editorial, Hlatky said the design of JUPITER provides only limited information about the role of CRP testing in clinical practice, since investigators did not compare subjects with and without CRP measurements and did not compare the use of CRP with the use of other markers of cardiovascular risk.

At this point, the current guidelines for measurement of high-sensitivity CRP remain reasonable. Measurements of CRP may be obtained in asymptomatic individuals who have an intermediate level of risk, based on standard clinical risk markers, and in whom treatment might change depending on the high-sensitivity CRP level. The use of more widespread screening of CRP values needs more investigation. The test has high variability and is elevated with infections and injuries, so abnormally high CRP levels do not always reflect arterial injury or cardiovascular-disease risk.

**References**


The most common omega-3 fatty acids contain 18-22 carbons and a signature double bond at the third position from the methyl (or n, or omega) end of the molecule (figure 1). These fatty acids must be obtained in the diet as they cannot be synthesized by vertebrates. They include the plant-derived alpha-linolenic acid (ALA, 18:3n-3), and the fish-oil-derived eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3).

**Figure 1. Structure of omega-3**

![Structure of omega-3 fatty acid](image)

**Table 1. Cardio-vascular benefit of fish oil**

<table>
<thead>
<tr>
<th>Studies</th>
<th>Type of intervention</th>
<th>Patients</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>DART</td>
<td>Fish 2x/week versus no R/</td>
<td>2033 post-myocardial infarctus</td>
<td>2 years</td>
<td>- 27% fatal MI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 29% Mortality</td>
</tr>
<tr>
<td>GISSI-Prevezione</td>
<td>1 g/J n-3 AGPI + fish versus placebo</td>
<td>11324 post-myocardial infarctus (&lt;3 mois)</td>
<td>3.5 years</td>
<td>- 20% Mortality</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 30% CV Mort.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 46% Sudden death</td>
</tr>
<tr>
<td>JELIS</td>
<td>1.8 g/J EPA and statin versus statin seule</td>
<td>18645 hypercholesterolemic patients</td>
<td>4.5 years</td>
<td>(NS) CV &amp; global Mortality</td>
</tr>
<tr>
<td>SOFA</td>
<td>2 g/J fish oil versus sunflower oil</td>
<td>546 with myocardial ischemia</td>
<td>1 year</td>
<td>Neutral</td>
</tr>
<tr>
<td>Burr et al.</td>
<td></td>
<td>3114 Men with pectoris angor</td>
<td>9 years</td>
<td>_ Sudden death</td>
</tr>
<tr>
<td>Raitt et al.</td>
<td>2 g/J fish oil versus olive oil</td>
<td>200 with defibrillator indicated for ventricular tachycardie/fibrillation, ejection fraction = 36%</td>
<td>2 years</td>
<td>_ recurrence of ventricular fibrillations</td>
</tr>
</tbody>
</table>
(EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). From a cardiovascular standpoint, the important dietary n-3 PUFAs include EPA and DHA. Normally, very little ALA is converted to EPA, and even less to DHA, and therefore direct intake of the latter two is optimal. The interest in n-3 PUFAs dates back to the late 1970s, when Dyerberg and coworkers demonstrated low rates of coronary events in Greenland Eskimos whose diet consisted mainly of fish and seal. The most compelling evidence for the cardiovascular benefit provided by omega-3 fatty acids comes from 3 large controlled trials of 32,000 participants randomized to receive omega-3 fatty acid supplements containing docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) or to act as controls (DART, GISSI, and JELI, see table 1) (Descamps 2009). Here is an update of the recommendations regarding the dosage to use in practice in various clinical situations, based on the recent literature and various presentations at the AHA meeting in New Orleans.

In the general population, 250 mg/d of EPA and DHA is recommended to reduce CHD mortality. The evidence base supports however a dietary recommendation of approximately 500 mg/d of EPA and DHA for cardiovascular disease risk reduction (Gebauer 2006). The consumption of equal amounts of EPA and DHA from oily fish on a weekly basis (2 fish meals per week, preferably fatty fish like salmon and albacore tuna) or from fish-oil capsules on a daily basis (1-2 capsules/d) is equally effective at enriching blood lipids with n-3 FAs (Harris 2007). Indeed, dietary n-3 PUFAs persist for weeks in tissue membranes.

For treatment of existing cardiovascular disease, 1 g/d is recommended. This could be approximated by one 6-oz serving/wk of fish richest in n-3 PUFAs (eg, farmed salmon, anchovies, herring), more frequent consumption of other fish. Alternative like, foods enriched with EPA and DHA or fish oil supplements may be necessary to achieve intakes of 1 g/d. Data from the GISSI-Prevenzione trial had suggested that 1g/d fish oil supplements led to a significant reduction in the risk of sudden cardiac death among patients with a recent MI: 9% in intention to treat analysis but 14% taking into account only the compliers. Such intake has no effect of lipids (only a 10mg/dL reduction of TG).

A mechanism that has been first proposed was that n-3 PUFAs may reduce the risk of fatal arrhythmias. However, ulterior studies (Study on Omega-3 Fatty Acid and Ventricular Arrhythmia (SOFA) and study in implantable cardioverter-defibrillators placed for sustained ventricular tachycardia or ventricular fibrillation) showed that omega-3 was neutral on sudden death or even proarrhythmic. This may suggest that the previously demonstrated beneficial effects of fish oil on sudden cardiac death in GISSI may thus not be due to the suppression of ventricular tachycardia or fibrillation and that, alternatively, any antiarrhythmic effect of fish oil may be most profound in the setting of acute ischemia or recent MI and not outside this setting (when ventricular tachycardia occurs as a result of myocardial scar-based reentry).

More recently, a large trial was also performed in patients with chronic heart failure (NYHA class II-IV, irrespective of cause and left ventricular ejection fraction), randomly assigned to n-3 PUFA 1 g daily (n=3494) or placebo (n=3481) (Tavazzi 2008). During the 3.9 years follow up, death occurred in 27% patients in the n-3 PUFA group and in 29% in the placebo group (HR=0.91, p=0.041). Death or admission to hospital for cardiovascular reasons occurs in 57% patients in the n-3 PUFA group

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**Table 2. Recommendations (adapted by Harris)**

<table>
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<th>Patient category</th>
<th>Recommendations</th>
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| Patients without coronary heart disease | 500 mg/day of EPA and DHA.  
- consumption of 2 fish meals per week (salmon …)  
- Or fish-oil capsules on a daily basis (1-2 capsules/day) |
| Patients without coronary heart disease | 1 g/day of EPA and DHA.  
- consumption of one 6-oz serving/week of fish richest in n-3 PUFAs (farmed salmon, anchovies, herring),  
- eventually foods enriched with EPA and DHA  
- Fish oil supplements may be necessary under medical control. |
| Patients with hypertriglyceridemia | 3-4 g/day of EPA and DHA.  
Fish oil supplements are necessary under medical control. |
and in 59% patients in the placebo group (HR 0.92, p=0.009). This demonstrated also that treatment with n-3 PUFA can provide a small beneficial advantage in terms of mortality and admission to hospital for cardiovascular reasons in patients with heart failure.

**A higher dosage, fish oil is also able to reduce hypertriglyceridemia.** The lowering of triglyceride levels is accomplished by decreasing the production of hepatic triglycerides and by increasing the clearance of plasma triglycerides.

In patients with triglyceride concentrations between 500 and 2000 mg/dl, 4 g omega-3/day significantly reduced triglyceride concentrations by 45% and cholesterol by 15% with an increased of HDL-C by 13%. Although in this study, LDL-C was increased by 31%, we should take into account that baseline LDL-C was around 89 mg/dL (Harris 1997).

In a more recent study of adding prescription omega-3 fatty acids 4 g/d to simvastatin 40 mg/d in hypertriglyceridemic (TG 250-499 mg/dL) patients, omega-3 was associated with significant reductions in TG (30% vs 6%) and a significant increase the clearance of plasma triglycerides.

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### Table 3. Ways to Get 1 g/d EPA+DHA

- **Fish**
  - 2–3 oz salmon, sardines, mackerel per day

- **Dietary Supplements**
  - *Low Potency*: 300 mg EPA+DHA/caps
    (Typical drug store capsules; 3 caps/day)
  - *Mid Potency*: 500–700 mg EPA+DHA/caps
    (Mail-order, online, etc; 2 caps/day)

- **Drugs**
  - *High Potency*: 850 mg EPA+DHA/g
    (Omega-3 acid ethyl esters; OMACOR®; 1 caps/day)

- **Cod Liver Oil**
  - 1 teaspoon (RDA for vitamin D; 2x RDA for vitamin A)
in HDL-C (3% vs -1%); (all, P < 0.001 vs placebo) (Figure 2). The combination therapy with omega-3 fatty acids and a statin is thus a safe and effective way to improve lipid levels and could also better the cardiovascular prognosis (not yet proven clinically, however) beyond the benefits provided by statin therapy alone.

Others

EPA and DHA and their metabolites have many biologic effects on membranes, eicosanoid metabolism, and gene transcription. Other effects of fish oil have been described, such as lowering heart rate and blood pressure, decreasing platelet aggregation. These effects occur only at high dosages, (Figure 3) and are considered as negligible in practice (Mozaffarian 2006).

In the future, blood DHA and EPA levels could be used to identify patients with deficient levels and to individualize therapeutic recommendations (Lee 2008).

Conclusions :

There is compelling evidence for the cardiovascular benefit provided by omega-3 fatty acids. These trials showed reductions in cardiovascular events of 19% to 45%. Currently, it is recommended that the intake of omega-3 fatty acids, whether from dietary sources or fish oil supplements, should be increased, especially in those with or at risk for coronary artery disease. Patients should consume both DHA and EPA. The target DHA and EPA consumption levels are about 1 g/d for those with known coronary artery disease and at least 500 mg/d for those without disease. Patients with hypertriglyceridemia benefit from treatment with 3 to 4 g/d of DHA and EPA, a dosage that lowers triglyceride levels by 20% to 50%. Although 2 meals of oily fish per week can provide 400 to 500 mg/d of DHA and EPA, secondary prevention patients and those with hypertriglyceridemia must use fish oil supplements if they are to reach 1 g/d and 3 to 4 g/d of DHA and EPA, respectively.

References:


