Eur J Nutr (2007) 46:103–110
DOI 10.1007/s00394-006-0638-3

ORIGINAL CONTRIBUTION

Zuyuan Xu
Natalie Riediger
Sheila Innis
Mohammed H. Moghadasian

Fish oil significantly alters fatty acid profiles in various lipid fractions but not atherogenesis in apo E-KO mice

Received: 13 June 2006
Accepted: 7 December 2006
Published online: 16 January 2007

Z. Xu · N. Riediger · M.H. Moghadasian
Dept. of Human Nutritional Sciences
University of Manitoba
Winnipeg (MB), Canada

Z. Xu · N. Riediger · M.H. Moghadasian
Canadian Centre for Agri-food Research in Health and Medicine
St. Boniface Hospital Research Centre
351 Tache Avenue
Winnipeg (MB), Canada

S. Innis
Dept. of Peadiatrics
University of British Columbia
Vancouver (BC), Canada

Dr. M.H. Moghadasian (✉)
351 Tache Avenue
Winnipeg (MB) R2H 2A6, Canada
Tel.: +1-204/235-3934
Fax: +1-204/231-1151
E-Mail: mmoghadasian@sbrc.ca

Abstract *Background* Consumption of fish oil and n-3 fatty acids is associated with beneficial modifications in plasma lipid levels. The impact of these modifications on development of atherosclerotic lesions merits further investigation. *Aim of the study* The aim of this study was to investigate the impact of fish oil consumption on quality and quantity of lipoprotein fatty acids and its influence on atherosclerosis in apolipoprotein E-knockout (apo E-KO) mice. *Methods* Male apo E-KO mice were treated with 1% dietary fish oil for 14 weeks. Plasma triglycerides (TG), phospholipids, (PL) and cholesteryl ester (CE) fractions were separated using thin layer chromatography. Plasma-free fatty acids (FFA) plus fatty acid contents of TG, PL, CE were determined using gas chromatography. Aortic atherosclerosis was assessed by histological and morphometrical techniques. *Results* Twenty-eight fatty acids were identified in each of the four lipid compartments. High amounts of n-3 fatty acids (eicosapentaenoic (EPA), docosahexaenoic (DHA)) were found in all of these fractions. The levels of EPA and DHA increased by 400 and 150%, respectively, in FFA, TG and PL compartments; higher increases (>500 and 200%) in EPA and DHA were found in CE. This markedly decreased the n-6/n-3 ratios in FFA, TG, PL, and CE by 60, 72, 53, and 61%, respectively. These changes were accompanied by a significant increase in plasma triglyceride levels. Surprisingly, these changes did not affect atherogenesis. *Conclusions* Elevated levels of EPA and DHA do not appear to prevent development of atherosclerotic plaques in this model. Longer studies warrant investigation of the direct benefits of these fatty acids against myocardial damage as clinical consequences of advanced atherosclerosis.

Key words apo E-KO mice – fish oil – fatty acids – triglycerides – cholesteryl ester – phospholipids

Introduction

Coronary artery disease (CAD) still remains as a leading cause for both morbidity and mortality in Western countries. Historically, significant attention has been given to elevated levels of low-density lipoprotein (LDL) cholesterol in the pathogenesis of CAD. However, recent studies highlighted the importance of high plasma triglyceride levels on the development of atherosclerotic vascular disease. For example, a meta-

analysis by Hokanson and Austin [1] showed a significant positive association between elevated levels of plasma triglycerides and atherosclerotic events. Similarly, plasma triglyceride levels were identified as an independent predictor for ischemic heart disease in men [2]. Thus, reducing plasma triglyceride levels by dietary and/or pharmacological agents is recommended for prevention and/or treatment of CAD. Among dietary agents, triglyceride-lowering effects of fish oil have been well-established in both humans and animal models [3–5]. The nature of fatty acid components of the triglycerides is probably the major contributing factor in its atherogenic properties. Particularly, saturated fatty acids may promote atherosclerosis, while long chain n-3 fatty acids are believed to prevent atherosclerosis.

Apoliporotein E plays a crucial role in triglyceride-rich lipoprotein metabolism. Subjects with apolipoprotein E-deficiency develop type III dyslipidemia with elevated levels of plasma triglycerides [6]. These subjects also develop atherosclerotic vascular disease. Similar to humans, we have shown that deletion of apolipoprotein E gene in mice is associated with significant increases in the level of plasma triglycerides and cholesterol [7]. However, other studies have shown that fatty acid composition in several lipid fractions of the brain of apolipoprotein E-knockout (apo E-KO) mice is comparable to that of their wild-type counterparts [8]. This suggests that deletion of apo E may not significantly change tissue fatty acid metabolism. These apo E-KO mice develop spontaneous atherosclerosis in their early stage of life [9]. Despite its extensive use, this animal model has certain limitations, which may question its application in studying human diseases. For example, rodent lipoprotein metabolism and atheroma formation are quite different of the human mechanisms. Therefore, one may prefer rabbit models over rodent models for studying the effects of fish oil on dyslipidemia and atherosclerosis. Regardless, we and others have shown that reductions in plasma cholesterol levels reduce the extent and severity of atherogenesis in this animal model [10–12]. Unlike cholesterol, the role of increased levels of plasma triglycerides in accelerated atherogenesis in apo E-KO mice has not been fully studied. Similarly, the nature of lipoprotein fatty acid profile and its contribution to accelerated atherogenesis in this animal model is not known.

Long chain n-3 fatty acids such as eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) and to a lesser extent alpha-lionlenic acid (ALA, C18:3n-3) are believed to be cardiovascular protective. Cardiovascular benefits from these fatty acids are not limited to their triglyceride-lowering properties. Anti-thrombotic, anti-arrhythmic, and anti-hypertensive effects of fish oil may also contribute to its preventive effects on both CAD and stroke. Many of these beneficial effects of fish oil are attributed to the type of n-3 fatty acids (EPA and DHA) found in fish, shellfish and marine mammals. In addition to several epidemiological studies, a recent study has reported a significant inverse association between intakes of EPA and DHA with carotid intimal-medial thickness in a total of 1902 subjects [13]. These epidemiological and observational studies were the bases of recommendations for increased intake of EPA and DHA by several medical authorities worldwide [14, 15]. However, available literature lacks documentation of the distribution of these fatty acids in various lipoprotein fractions and their relation to atherosclerosis. Thus, the aim of this study was (a) to characterize fatty acid profile of various plasma lipid fractions in apo E-KO mice with severe dyslipidemia and atherosclerosis, and (b) to investigate to what extent the n-3 fatty acids of fish oil are incorporated to various plasma lipid fractions, and how this affects the overall plasma/lipoprotein fatty acid profile and atherogenesis in this model of dyslipidemia and atherosclerosis. Simultaneously, the association between plasma triglyceride levels and the extent of atherosclerosis was studied.

Materials and Methods

Animals and diets

Fifteen male 4-week-old apo E-KO mice were purchased from Jackson Laboratories, Ann Harbor, ME. After a 10-day adaptation period, the animals were divided into two groups (treated, $n = 8$ and controls, $n = 7$) matched with their average body weight and plasma total cholesterol levels as previously described [10, 11]. PicoLab mouse chow containing 9% (w/w) fat was supplemented with 0.2% (w/w) cholesterol to generate "control" diets. This diet was further supplemented with 1% fish oil (Pronova Biocare, Sandefjord, Norway). The animals fed the experimental diets for 14 weeks; body weights were recorded weekly. Blood samples were taken at baseline and 4-week intervals. To determine the contribution of various fatty acids (without dietary supplementation) in lipoprotein structures and their relation to atherogenesis and subsequently the role of dietary EPA and DHA in these parameters, addition of a non-n-3 fatty acids to the diet of the control group was avoided. At the end of the study, the animals were sacrificed under deep anesthesia (60 mg/kg pentobarbital i.p.) and final blood samples were taken by cardiac puncture as previously described [10, 11]. The studies were approved by the Animal Care Committee at the University of Manitoba, Winnipeg, Canada.

Lipid analysis

Plasma was separated and used for determination of cholesterol and triglyceride concentrations using standard enzymatic assays [10, 11]. Plasma cholesteryl ester, triglyceride and phospholipid fractions were separated using thin layer chromatography as previously described [16]. Plasma free fatty acid profile and fatty acid composition of the above-mentioned lipid fractions were determined using gas chromatography techniques [16].

Histology and morphometry

The hearts were harvested and fixed in 10% formalin. Sections from aortic roots were cut and stained with oil red O, hematoxylin and eosin and Movat's pentachrome for histological and morphometrical examinations. A series of oil red O-stained sections were used for morphometrical analysis using image ProPlus analysis system as previously described [11].

Statistical analysis

The two-tailed student-*t* test was used to determine the significant differences between the control and treated animals at the level of $P < 0.05$. We also used SPSS software version 11.5 for Windows® to test normal distribution of our data at baseline and at the end of the study. Normal distribution was confirmed when the standard error of skewness was greater than two times the skewness values. Our analysis of body weight and plasma cholesterol concentrations at baseline revealed normal distribution. Similarly, the levels of major fatty acids, including ALA, EPA, DHA, oleic acid and linoleic acid in various fractions of plasma lipids as well as body weight at the end of the study revealed normal distribution. Data are expressed as mean ± standard deviation.

Results

Body weight

Figure 1 shows body weight gain for the control and fish-oil-treated groups over the experimental course. Both groups of mice had similar body weight gains. This indicates that addition of 1% fish oil to the diet did not result in higher body weight in the treated group. Both groups of animals had the highest rate of body weight gain (approximately 25%) during the first two weeks of the study. The extent of body weight gain was lower from week 4 to week 8; it basically reached plateau after week 8 in both groups of mice (Fig. 1).

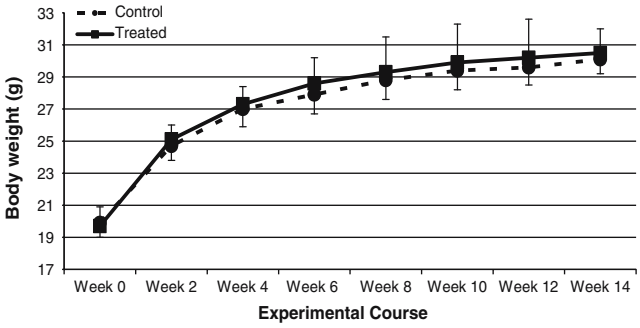

Fig. 1 Body weight gain in the control and fish-oil-treated apo E-KO mice over 14 weeks of experimental course. *Broken line*, controls; *solid line*, fish-oil-treated

Plasma total cholesterol and triglycerides

Plasma total cholesterol and triglyceride levels at baseline and during the experimental course are shown in Figs. 2 and 3. Both groups of mice had similar cholesterol levels (460 vs 465 mg/dl) at baseline; these levels increased approximately 3 times (460 vs. 1250, mg/dl) in both groups by week 4, an expected outcome of the ingestion of dietary cholesterol through the experimental diet (Fig. 2). The levels of total cholesterol remained steady and comparable between the two groups over the rest of experimental course (Fig. 2). In contrary, the levels of plasma triglyceride levels significantly increased (65 vs. 210, mg/dl) in fish-oil-treated animals at week 4 and remained high throughout the study as compared to controls (Fig. 3).

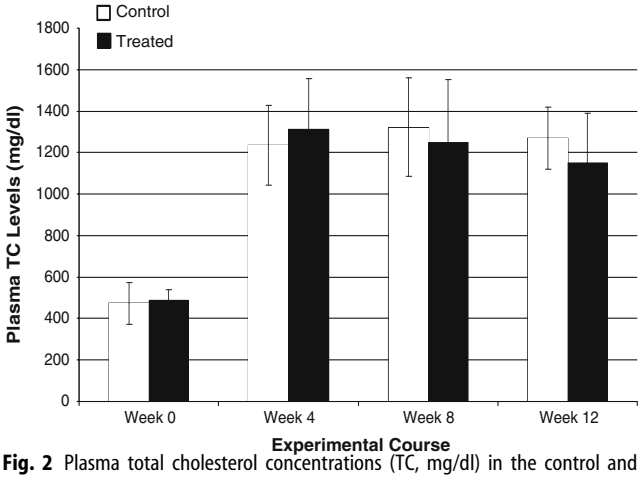

Fig. 2 Plasma total cholesterol concentrations (TC, mg/dl) in the control and fish-oil-treated apo E-KO mice over the experimental course. Dietary cholesterol significantly increased plasma cholesterol levels, but fish oil treatment had no effects on total cholesterol concentrations. *Open bars*, controls; *solid bars*, fish-oil-treated

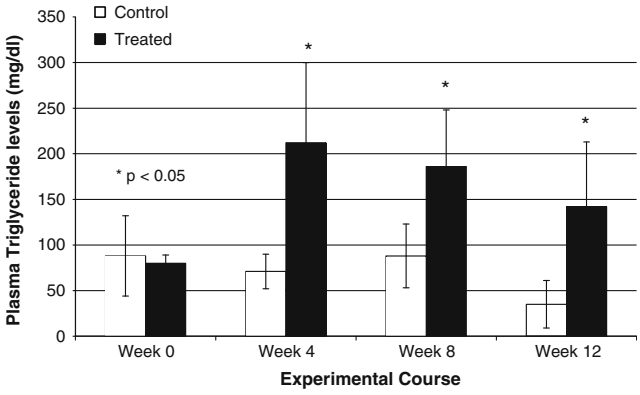

Fig. 3 Plasma triglyceride concentrations (TG, mg/dl) in the control and fish-oil-treated apo E-KO mice over the experimental course. Fish oil treatment significantly increased plasma triglyceride levels by week 4 of the study, as compared to controls. *Open bars*, controls; *solid bars*, fish-oil-treated; *, $P < 0.05$

Fatty acid profiles

A complete fatty acid analysis was performed on the final plasma samples. Table 1 summarizes the composition of fatty acids in each fraction of free fatty acid, cholesteryl ester, triglycerides, and phospholipids from the two groups of control and fish-oil-treated apo E-KO mice. In the control group, fatty acids C16:0, C18:1n-9, and C18:2n-6 were among the most abundant fatty acids in plasma free fatty acid pool. C18:3n-3, C20:4n-6, C20:5n-3, C22:0, and C22:6n-3 were found in free fatty acid pool in intermediate amounts. In triglyceride fraction, C16:0, C18:1n-9, C18:2n-6, C20:5n-3, and C22:6n-3 were in high amounts, while C16:1n-7, C18:0, C18:1n-7, C18:3n-3, C20:4-n6, C22:0 were in intermediate amounts. High amounts of C16:0, C18:0, and C18:2n-6, and intermediate amounts of C18:1n-9, C18:1n-7, C20:3n-6, C20:4n-6, and C20:5n-3 were found in phospholipids. C16:0, C18:1n-9, and C18:1n-7 were found in high amounts in cholesteryl ester fraction. Other detectable fatty acids were found to be in very small amounts in all of the four plasma lipid compartments. In the treated group, 14 weeks on fish oil (1% w/w) substantially changed the fatty acid profile in all of the four lipid fractions. For example, the levels of EPA and DHA increased by 400 and 150%, respectively, in free fatty acid, triglyceride and phospholipid compartments; higher increases (>500 and 200%) in EPA and DHA contents were found in cholesteryl ester. In addition to EPA and DHA, the levels of several other polyunsaturated fatty acids in the lipid fractions were

Table 1 Plasma fatty acid profile in various fractions in the fish-oil-treated and control apo E-KO mice at the end of the study (means; pmol/mg lipid extract; controls ($n = 7$), treated ($n = 8$).

Fatty acids	Free fatty acids		Triglycerides		Phospholipids		Cholesteryl ester	
	Controls	Treated	Controls	Treated	Controls	Treated	Controls	Treated
C12:0	0.17	0.11	0.00	0.01	0.00	0.00	0.01	0.02
C14:0	0.82	0.71	0.27	0.22	0.10	0.12	0.30	0.30
C14:1	0.40	0.50	0.07	0.11	0.01	0.01	0.06	0.05
C16:0	31.4	33.8	17.9	10.0*	25.5	26.8	17.6	17.7
C16:1n9	0.48	0.35*	0.51	0.30*	0.13	0.13	0.32	0.29
C16:1n7	1.91	1.31*	1.25	0.82*	0.29	0.25	3.15	2.68*
C18:0	12.9	12.5	5.77	2.45*	17.7	17.8	6.99	7.05
C18:1n9	16.9	11.9*	22.9	14.1*	6.81	6.79	32.1	30.2
C18:1n7	1.16	0.69*	1.09	0.57*	1.35	1.00*	1.38	1.51
C18:2n6	18.4	15.1*	20.7	15.3*	30.24	25.6*	28.4	24.6
C18:3n6	0.17	0.10*	0.33	0.13*	0.09	0.07*	0.24	0.09
C18:3n3	1.03	0.88	1.25	0.90*	0.18	0.14*	2.43	2.51
C20:0	0.87	0.86	0.55	0.17*	0.34	0.38*	0.17	0.16
C20:1	0.09	0.08	0.25	0.29	0.05	0.04	0.09	0.14
C20:2n6	0.82	0.44	0.47	0.16*	0.36	0.27*	0.10	0.10
C20:3n9	0.59	1.08	0.60	0.27*	0.08	0.04*	0.13	0.13
C20:3n6	0.55	0.50	0.45	0.15*	1.57	0.93*	0.16	0.14
C20:4n6	1.65	1.36	4.82	3.67*	6.29	4.76*	2.76	2.29
C20:5n3	1.28	6.00*	8.9	38.7*	1.25	5.11*	0.98	5.34*
C22:0	1.97	2.81*	1.20	0.37*	0.27	0.34	0.07	0.07
C22:1n11	0.023	0.07	0.11	0.12	0.04	0.07*	0.04	0.10*
C22:1n9	0.11	0.14	0.23	0.24	0.06	0.14*	0.05	0.12*
C22:4n6	0.10	0.03*	0.14	0.14	0.16	0.07*	0.02	0.05
C22:5n6	0.05	0.08	0.19	0.22	0.07	0.14	0.02	0.06*
C22:5n3	0.45	1.03*	0.67	1.58	0.47	0.84*	0.08	0.24*
C22:6n3	3.90	5.78*	7.69	8.21	5.48	6.90*	1.56	3.12*
C24:0	0.71	0.80	0.26	0.08*	0.12	0.15	0.00	0.00
C24:1	0.08	0.03	0.03	0.01	0.43	0.45	0.01	0.01

*, $P < 0.05$ as compared with controls

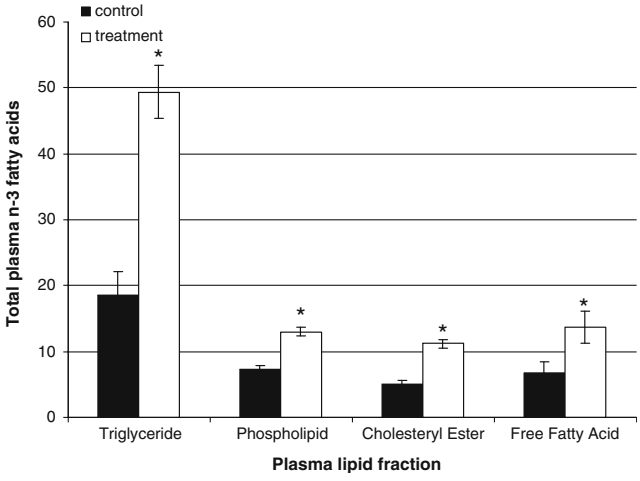

Fig. 4 Plasma total n-3 fatty acid concentrations (pmol/mg lipid extract) in various lipid fractions at the end of the study. Fish oil treatment significantly increased plasma total n-3 fatty acid contents of all four lipid fractions, as compared to controls. *Solid bars*, controls; *open bars*, fish-oil-treated; *, $P < 0.05$

significantly changed after fish oil consumption. Figure 4 shows that fish oil treatment significantly increased total n-3 fatty acid contents of all lipid fractions examined, as compared to controls. Furthermore, the treatment resulted in substantial changes in n-6 to n-3 ratios in all of the lipid fractions studies (Table 2). After fish oil treatment, this ratio reduced by 60% (from 3.22 to 1.28), 72% (from 1.44 to 0.40), 53% (from 5.25 to 2.45), and 61% (from 6.28 to 2.43) in free fatty acid pool, triglycerides, phospholipids, and cholesteryl ester, respectively. In contrary, reduced levels of several mono and polyunsaturated fatty acids such as C18:1n9, C18:1n-7, C18:2n-6, C18:3n-6, C18:3n-3, C20:2n-6, C20:3n-9, C20:3n-6, and C20:4n-6 were observed in various lipid fractions in the treated animals as compared to controls (Table 1). Fish oil did not alter the levels of medium-chain saturated fatty acids (C12:0, C14:0, and C16:0) in any of the four lipid fractions studied. Similarly, minimal effects of fish oil were observed on the levels of long-chain saturated fatty acids (C20:0, C22:0, and C24:0).

Table 2 The overall ratio of n-6 to n-3 fatty acids in various lipid fractions in apo E-KO mice and the effects of long-term fish oil treatment on this ratio

Lipid Fractions	Overall n-6 to n-3 fatty acid ratio		
	Control ($n = 7$)	Fish-oil-treated ($n = 8$)	Reductions
Free fatty acid	3.22	1.28	−60%
Triglycerides	1.44	0.40	−72%
Phospholipids	5.25	2.45	−53%
Cholesteryl ester	6.28	2.43	−61%

Morphological and morphometrical assessments of atherosclerotic lesions

Histological examinations of serial transverse sections from the aortic roots revealed similar features of atherosclerotic lesions in both groups of animals. The atherosclerotic lesions in both groups were mainly consisted of neutral lipids as demonstrated by oil red O staining. Similarly, numerous cholesterol clefts along with comparable amount of cellular and extracellular components of atherosclerotic lesions were observed in both control and treated animals. Overall, pathological features of these lesions resembled those observed in the control groups of our previous studies [9, 10]. For this reason and because we have extensively shown photomicrographs of such lesions [7, 9, 10], illustration of the atherosclerotic lesions is not included in this report. As it was evident in histological examinations, morphometrical analysis also showed very similar atherosclerotic lesion size in the two groups of animals (30 vs. 29 mm^2).

Discussion

In this study, the fatty acid profile of plasma cholesteryl ester, triglycerides, and phospholipids as well as plasma free fatty acid pool in apo E-KO mice has been characterized. Furthermore, the impact of fish oil consumption on the fatty acid compositions of the above-mentioned lipid fractions was studied. Finally, the influence of EPA and DHA on atherosclerosis was examined. Ingestion of a diet enriched with fish oil substantially altered fatty acid compositions of the four plasma lipid fractions (free fatty acids, cholesteryl ester, phospholipids, and triglycerides). These changes were accompanied by an increase in plasma triglyceride concentrations and no effects on plasma total cholesterol levels or the extent of aortic atherosclerosis in this animal model.

Numerous human and animal studies have shown triglyceride-lowering, anti-arrhythmic, anti-thrombotic, and anti-inflammatory effects of fish oil. These features are most likely the underlying mechanisms of cardiovascular protective properties of fish oil observed in humans and experimental animals. Convincing evidence for protection against cardiovascular diseases is provided by randomized clinical trials. However, direct evidence for anti-atherogenic properties of fish oil has not been fully documented yet. Recently, Hino et al. [13] have reported an inverse association between fish intake and intima-media thickness (IMT), a marker of carotid atherosclerosis. The present study failed to reveal such association in apo E-KO mice. Long-term (14 weeks) treatment with fish oil did not reduce the quality or quantity of

atherosclerotic lesions in the aortic valves. Interestingly, fish oil treatment was accompanied by a marked increase in plasma triglyceride levels, which may suggest that increased plasma triglyceride concentrations are not atherogeic in this model. Moreover, high amounts of n-3 fatty acids, particularly EPA and DHA in lipid fractions were not sufficient to prevent atherosclerosis. On the other hand, comparable concentrations of plasma total cholesterol and atherosclerotic lesion size between the treated group and the control group may suggest that plasma cholesterol levels are major contributing factors for the development of atherosclerotic lesions in this model. Our previous studies in which reductions in plasma cholesterol levels by phytosterols resulted in prevention of atherosclerosis support this notion.

The present data on plasma triglyceride levels and atheromatous vascular disease in apo E-KO mice add to the controversial efficacy of fish oil reported from epidemiological and observational studies. For example, Stone [17] reported a lower CAD mortality rate in men who ate fish as compared to men who did not. Zhang et al. [18] reported reduced risks of CAD and stroke mortality due to fish consumption across 36 countries. Furthermore, a dose-response relationship was reported between the frequency of fish intake and reduced cardiovascular risk factors in Japanese subjects [19]. Similarly, the Nurses' Health Study reported a negative association between the amount of fish and n-3 fatty acid consumption and CAD death [20]. Low cardiovascular mortality in Eskimos is believed to be associated with consuming a traditional diet high in EPA and DHA [21]. Similarly, an inverse correlation between the intake of EPA and DHA and total or cardiovascular mortality rate was found in Western populations [22]. These observations led the American authorities to recommend an increase in fish intake by general population.

In contrary, other studies failed to reproduce such association. For example, the Health Professionals' Follow-up Study [23] showed no relation between fish or n-3 fatty acid intake with risk of fatal coronary disease or non-fatal myocardial infarction or coronary artery bypass grafting. Likewise, the US Physicians' Health Study [24] did not show an association between fish and n-3 fatty acid intake and reduced risk of total myocardial infarction, non-sudden cardiac death, or total cardiovascular mortality. Two other reports, namely the EURAMIC Study (European Multicentre Case–Control Study on Antioxidants, Myocardial Infarction and Breast cancer Study) [25] and a meta-analysis of the Seven Countries data [26] also reported no association between fish consumption and the overall risk of cardiovascular mortality. Furthermore, Ebbesson et al. [27] reported that up to 26% of a cohort of 454 Alaskan Eskimos aged 55 or older had coronary heart disease despite high intake of n-3 fatty acids and high levels of plasma n-3 fatty acids. However, in another study these investigators reported that high intakes of n-3 fatty acids in Alaskan Eskimos may protect them against metabolic syndrome and glucose intolerance [28]. The above-mentioned contrasting data regarding benefits of n-3 fatty acids against CAD may reflect differences in study populations and individual genetic and metabolic background.

One possible mechanism of protection against cardiac mortality is the incorporation of the n-3 fatty acid into the myocardium, plasma lipoproteins and other cellular compartments. Harris et al. [29] have shown that long-term fish oil supplementation results in the incorporation of n-3 fatty acids in various tissues (cardiac and buccal cells, red cells and plasma) in humans. Other studies [30–32] report that (a) plasma phospholipids fatty acids are closely related to that of cardiac tissue, and (b) that n-3 fatty acids in phospholipids reflect fish intake in humans. Thus, the whole plasma fatty acid profile may not be a good representative of either tissue n-3 fatty acid levels or fish consumption. The present study reports substantial alterations in the fatty acid compositions of plasma phospholipids and triglyceride fractions after long-term ingestion of fish oil in apo E-KO mice.

Despite triglyceride-lowering effects of fish oil in humans or wild-type animals, fish oil does not reduce triglyceride levels in apo E-KO mice. Indeed, the present study reports paradoxical effects of fish oil with this regard. Consumption of fish oil was associated with significant increases in plasma triglyceride levels (Fig. 3). This paradoxical effect of fish oil may be explained, at least in part, by the role of apo E on metabolism of fatty acids and triglyceride-rich lipoprotein particles. Apo E is needed for clearance of these triglyceride-rich particles, and therefore, a lack of apo E may be responsible for the lack of triglyceride-lowering effects of fish oil in apo E-KO mice. We have previously reported that VLDL is the prominent lipoprotein particle in plasma of apo E-KO mice [10]; this lipoprotein particle accumulates in plasma due to a lack of apo E-mediated uptake of these particles. We now speculate that higher levels of n-3 fatty acids in plasma further impair this apo E-mediated pathway, resulting in additional accumulation of triglyceride-rich particles in plasma. It is possible that a lack of apo E contributes to an increase in hepatic synthesis of triglyceride-rich particle by n-3 fatty acids as well as a simultaneous reduction in their catabolism. However, our data suggest that apo E may not have a role in absorption or distribution of n-3 fatty acids, as a significant rise in the levels of n-3 fatty acids in various fractions of plasma lipids was observed (Fig. 4). Our studies show that incorporation

of EPA and DHA in plasma lipid fractions including phospholipids, the best representative of tissue EPA/DHA pool, does not result in prevention of atherosclerotic lesions in apo E-KO mice. This observation may suggest that the cardiovascular benefits from these fatty acids are most likely mediated through mechanisms other than prevention of atherosclerosis. In agreement with this speculation, Leaf et al. [33] reported that reductions in plasma lipids and blood pressure do not appear to explain the mechanisms of benefits of EPA and DHA against sudden cardiac death. Numerous animal studies [34–37] strongly suggest that EPA and DHA have direct protective effects on the heart itself. These effects may be mediated through alterations in the dynamics of sodium and calcium channel function. It is proposed that ischemia-activated phospholipase A_2 releases EPA and DHA from myocardial membrane phospholipids, leading to alterations in ion channels. Therefore, enrichment of cardiac tissue with EPA and DHA may prevent the clinical consequences of atherosclerosis such as arrhythmias, but does not appear to prevent the development of atherosclerotic lesions. It would have been beneficial, if we could measure the levels of n-3 fatty acids in arterial wall and cardiac tissues. However, we used these tissues for assessment of atherosclerotic lesions.

In conclusion, we report high amounts of n-3 fatty acids in various lipoprotein fractions in apo E-KO mice with severe atherosclerosis. Further substantial enrichment of lipoproteins with EPA and DHA increases plasma triglyceride levels with no change in atherogenesis in this animal model. One reason for this paradoxical effect of fish oil could be the lack of apo E and the animal model used. It is possible that benefits from EPA and DHA are mediated through mechanisms other than prevention of atherosclerotic plaque formation. As fatty acid components of phospholipids are shown to be a true representative of tissue fatty acids and we observed high amount of EPA and DHA in phospholipids of fish-oil-treated mice, this study suggests that dietary fish oil may substantially increase myocardial contents of EPA and DHA. Thus, longer studies warrant investigation of the direct benefits of these fatty acids against myocardial damage and sudden death as clinical consequences of advanced atherosclerosis. As increased plasma triglyceride levels by fish oil did not affect atherogenesis, it may be concluded that elevated levels of plasma triglyceride may not substantially contribute to accelerated atherogenesis in apo E-KO mice.

Acknowledgements This study was supported in part by Canadian Institute of Health Research, Natural Sciences and Engineering Research Council of Canada and the Heart and Stroke Foundation of Canada to MHM. NR is an NSERC Graduate Student Fellowship recipient.

References

1. Hokanson JE, Austin MA (1996) Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 3:213–219
2. Jeppesen J, Hein HO, Suadicani P, Gyntelberg F (1998) Triglyceride concentration and ischemic heart disease: an eight-year follow-up in the Copenhagen Male Study. Circulation 97:1029–1036
3. Chan DC, Watts GF, Mori TA, Barrett PH, Redgrave TG, Beilin LJ (2003) Randomized controlled trial of the effect of n-3 fatty acid supplementation on the metabolism of apolipoprotein B-100 and chylomicron remnants in men with visceral obesity. Am J Clin Nutr 77:300–307
4. Sacks FM, Katan M (2002) Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am J Med 113 Suppl 9B:13S–24S
5. Ikeda I, Kumamaru J, Nakatani N, Sakono M, Murota I, Imaizumi K (2001) Reduced hepatic triglyceride secretion in rats fed docosahexaenoic acid-rich fish oil suppresses postprandial hypertriglyceridemia. J Nutr 131:1159–1164
6. Lohse P, Brewer HB 3rd, Meng MS, Skarlatos SI, LaRosa JC, Brewer HB Jr (1992) Familial apolipoprotein E deficiency and type III hyperlipoproteinemia due to a premature stop codon in the apolipoprotein E gene. J Lipid Res 33:1583–1590
7. Moghadasian MH, McManus BM, Nguyen LB, Hill J, Scudamore C, Frohlich JJ (2001) Pathophysiology of apolipoprotein E deficiency in mice: relevance to apo E-related disorders in humans. FASEB J 15:2623–2630
8. Lomnitski L, Oron L, Sklan D, Michaelson DM (1999) Distinct alterations in phospholipid metabolism in brains of apolipoprotein E-deficient mice. J Neurosci Res 58:586–592
9. Zhang SH, Reddick RL, Piedrahita JA, Maeda N (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 258:468–4671
10. Moghadasian MH, McManus BM, Godin DV, Rodrigues B, Frohlich JJ (1999) Proatherogenic and antiatherogenic effects of probucol and phytosterols in apolipoprotein E-deficient mice: possible mechanisms of action. Circulation 99:1733–1739
11. Moghadasian MH, McManus BM, Pritchard PH, Frohlich JJ (1997) "Tall oil"-derived phytosterols reduce atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 17:119–126
12. Davis HR Jr, Compton DS, Hoos L, Tetzloff G (2001) Ezetimibe, a potent cholesterol absorption inhibitor, inhibits the development of atherosclerosis in ApoE knockout mice. Arterioscler Thromb Vasc Biol 21:2032–2038

13. Hino A, Adachi H, Toyomasu K, Yoshida N, Enomoto M, Hiratsuka A, Hirai Y, Satoh A, Imaizumi T (2004) Very long chain n-3 fatty acids intake and carotid atherosclerosis: an epidemiological study evaluated by ultrasonography. Atherosclerosis 176:145–149
14. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD (2000) Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 71(1 Suppl):179S–188S
15. Kris-Etherton PM, Harris WS, Appel LJ, AHA Nutrition Committee, American Heart Association (2003) Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. Arterioscler Thromb Vasc Biol 23:151–152
16. Innis SM, Dyer RA (1999) Dietary canola oil alters hematological indices and blood lipids in neonatal piglets fed formula. J Nutr 129:1261–1268
17. Stone NJ (1996) Fish consumption, fish oil, lipids, and coronary heart disease. Circulation 94:2337–2340
18. Zhang J, Sasaki S, Amano K, Kesteloot H (1999) Fish consumption and mortality from all causes, ischemic heart disease, and stroke: an ecological study. Prev Med 28:520–529
19. Mizushima S, Moriguchi EH, Ishikawa P, Hekman P, Nara Y, Mimura G, Moriguchi Y, Yamori Y (1997) Fish intake and cardiovascular risk among middle-aged Japanese in Japan and Brazil. J Cardiovasc Risk 4:191–199
20. Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D, Manson JE (2002) Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 287:1815–1821
21. von Schacky C (1987) Prophylaxis of atherosclerosis with marine omega-3 fatty acids. A comprehensive strategy. Ann Intern Med 107:890–899
22. Dolecek TA (1992) Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. Proc Soc Exp Biol Med 200:177–182
23. Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL, Willett WC (1995) Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. N Engl J Med 332:977–982
24. Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE (1998) Fish consumption and risk of sudden cardiac death. JAMA 279:23–28
25. Guallar E, Aro A, Jimenez FJ, Martin-Moreno JM, Salminen I, van't Veer P, Kardinaal AF, Gomez-Aracena J, Martin BC, Kohlmeier L, Kark JD, Mazaev VP, Ringstad J, Guillen J, Riemersma RA, Huttunen JK, Thamm M, Kok FJ (1999) Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: the EURAMIC study. Arterioscler Thromb Vasc Biol 19:1111–1118
26. Kromhout D, Feskens EJ, Bowles CH (1995) The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. Int J Epidemiol 24:340–345
27. Ebbesson SO, Risica PM, Ebbesson LO, Kennish JM (2005) Eskimos have CHD despite high consumption of omega-3 fatty acids: the Alaska Siberia project. Int J Circumpolar Health 64:387–395
28. Ebbesson SO, Risica PM, Ebbesson LO, Kennish JM, Tejero ME (2005) Omega-3 fatty acids improve glucose tolerance and components of the metabolic syndrome in Alaskan Eskimos: the Alaska Siberia project. Int J Circumpolar Health 64:396–408
29. Harris WS, Sands SA, Windsor SL, Ali HA, Stevens TL, Magalski A, Porter CB, Borkon AM (2004) Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. Circulation 110:1645–1649
30. Harris WS, Von Schacky C (2004) The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 39:212–220
31. Harris WS (1989) Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J Lipid Res 30:785–807
32. Silverman DI, Reis GJ, Sacks FM, Boucher TM, Pasternak RC (1990) Usefulness of plasma phospholipid n-3 fatty acid levels in predicting dietary fish intake in patients with coronary artery disease. Am J Cardiol 66:860–862
33. Leaf A, Kang JX, Xiao YF, Billman GE (2003) Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646–2652
34. Culp BR, Lands WE, Lucches BR, Pitt B, Romson J (1980) The effect of dietary supplementation of fish oil on experimental myocardial infarction. Prostaglandins 20:1021–1031
35. McLennan PL, Bridle TM, Abeywardena MY, Charnock JS (1993) Comparative efficacy of n-3 and n-6 polyunsaturated fatty acids in modulating ventricular fibrillation threshold in marmoset monkeys. Am J Clin Nutr 58:666–669
36. McLennan PL (1993) Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. Am J Clin Nutr 57:207–212
37. McLennan PL, Abeywardena MY, Charnock JS (1988) Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. Am Heart J 116:709–717