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N-3 vs. Saturated fatty acids: Effects on the arterial wall

S. Sudheendran, **C. C. Chang**, and **R.J. Deckelbaum**
Department of Pediatrics and the Institute of Human Nutrition, College of Physicians and Surgeons, Columbia University, New York, NY10032, USA

Abstract

Cardiovascular disease is a leading cause of death world wide. Atherosclerosis and plaque instability are the underlying causes for most cardiovascular diseases. Cardiovascular disease is associated with consumption of diets high in saturated fats. In contrast there is increasing evidence that higher intakes of dietary n-3 fatty acids decrease risk for cardiovascular disease. Recent studies are beginning to clarify how n-3 compared to saturated fatty acids influence cardiovascular disease risk via pathways in the arterial wall. In this paper we will review studies which report on mechanisms whereby dietary fatty acids affect atherosclerosis through modulation of arterial wall lipid deposition, inflammation, and cell proliferation, and plaque vulnerability.

INTRODUCTION

Consumption of different dietary fatty acids can influence the risk and progression of cardiovascular disease via multiple pathways. High intakes of saturated fatty acids are associated with an increased risk of cardiovascular disease, whereas omega-3 (n-3) fatty acids have been linked to cardiovascular protection [1]. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the primary n-3 fatty acids with proven benefits, while there is less evidence for the cardiovascular benefits of the essential n-3 fatty acid α-linolenic acid [2,3]. N-3 fatty acids are present in fish as EPA and DHA in different concentrations and relative amounts, according to the type of fish and the diet of the fish [4,5].

The effects of n-3 fatty acids on mechanisms related to atherogenesis in the arterial wall are much different than those of saturated fatty acids. Pathways involved likely influence the risk of cardiovascular disease from early in atherogenesis to the final complications, such as thrombosis and atheroembolism. Fatty acids may influence lipid deposition in the arterial wall and overall lipid metabolism, along with modulating inflammatory processes. The pathways by which different fatty acids exert such effects have not been fully clarified but are of interest as greater knowledge and understanding of these mechanisms may contribute to therapeutic advances. In this paper, we will review recent studies that contribute to the understanding of mechanisms by which specific fatty acids affect the arterial wall through their modulation of lipid deposition, inflammation, cell proliferation, and plaque stability.

Correspondence to: R.J. Deckelbaum, Institute of Human Nutrition, College of Physicians and Surgeons, Columbia University, 630 W 168th Street, PH1512, New York, NY 10032, USA, Tel.: +1 212 305 4808, Fax: +1 212 305 3079. rjd20@columbia.edu.

ARTERIAL LIPID DEPOSITION

Fatty acids influence lipid deposition by influencing levels of circulating lipoproteins and their arterial uptake, efflux, and metabolism. Lipid deposition is an initial step in atherogenesis [6]. Lipid deposition begins with the entry of lipoproteins into the arterial wall, which initiates a proinflammatory cascade that attracts monocytes into the subendothelial space. Here the monocytes differentiate into macrophages that take up the lipoproteins, eventually becoming foam cells. These foam cells make up the fatty streak that is the beginning of an atherosclerotic plaque. Lipid deposition at the level of the arterial wall is reflective of the balance between lipoprotein uptake and efflux [7]. Arterial lipoprotein uptake is affected by the concentration of circulating lipoproteins and may be modulated by dietary fatty acids. Low-density lipoprotein (LDL) entering the arterial wall can be oxidized or modified and then taken up by macrophages in the arterial wall, advancing the process of lipid deposition and contributing to the inflammatory cascade.

Dietary fatty acids are incorporated into chylomicron remnants that likely influence the lipid accumulation with in an arterial macrophage [8,9]. When compared to remnants rich in n-3 fatty acids, chylomicron remnants rich in saturated fatty acids are taken up more rapidly by cultured macrophages and could result in greater arterial lipid accumulation [10]. De Pascale et al. reported that this difference in uptake and lipid accumulation was likely due to fatty acids modulating the activity of the transcription factor nuclear factor-kappa B (NFκB) in macrophages. In comparison to chylomicron remnants enriched with saturated fatty acids, those enriched with n-3 fatty acids resulted in lower NFκB activation and decreased production of tumor necrosis factor alpha (TNF-α). Chylomicron remnants enriched with n-3 fatty acids also resulted in increased cholesterol efflux from and decreased lipid accumulation in macrophages [11].

Lipid deposition in the innermost layer of the arterial wall, the intima, is associated with atherogenesis [12]. Binding and uptake of lipids in the arterial wall by macrophages is likely enhanced by arterial lipoprotein lipase (LPL) [13]. Rumsey et al. and others have shown the addition of LPL to incubations of LDL with macrophages results in a 10-fold increase in macrophage binding of LDL. a four fold increase in the uptake of LDL and a doubling of LDL degradation [14]. Thus LPL in addition to its catalytic activity on triglycerides, can also serve as a bridging or anchoring molecule for LDL and other lipoproteins to the macrophage cell surface [14].

In addition to whole particle uptake, selective uptake, which is the uptake of cholesteryl esters from LDL without the uptake of the whole LDL particle [15], leads to the accumulation of cholesterol within the cells and tissues, such as the arterial wall, and can contribute to atherogenesis. Seo et al. reported that a high saturated fat diet quadrupled arterial cholesterol delivery via total LDL and selective uptake in mice, and that this was associated with increases in arterial wall LPL activity [15,16]. Chang et al. again showed the increase in arterial uptake of LDL-cholesterol linked to increased arterial LPL expression on a high saturated fat diet. Mice fed a high saturated fat diet also had increased selective uptake of cholesteryl esters, higher LDL infiltration, and higher LDL cholesteryl ester deposition within the arterial wall. Mice fed a diet high in n-3 fatty acids had almost no selective uptake and much decreased whole particle LDL uptake. N-3 fatty acid fed mice had significantly decreased LPL within the aortic media although LPL was present within the intimal and subendothelial layers. The influence of n-3 fatty acids on LPL expression correlates with their effect on localization of lipid deposits to the intimal or subendothelial area. Thus n-3 fatty acids may protect the arterial wall by decreasing lipoprotein uptake and directing lipid deposition away from the aortic media by influencing LPL expression and distribution in the arterial wall [17].

Arterial LPL expression reflects macrophage LPL expression [18,19]. Macrophage LPL expression may be increased by saturated fatty acids and decreased by n-3 fatty acids [18]. Michaud et. al reported that macrophage LPL may be increased by saturated fatty acids via gene transcription in a PPAR-dependent manner [20]. In DNA binding assays, nuclear proteins from macrophages incubated with saturated fatty acids had enhanced binding to the peroxisome proliferator response element (PPRE) consensus sequence on the LPL promoter gene. In the nuclear proteins from macrophages incubated with saturated fatty acids, this enhanced binding activity was diminished by immunoprecipitation with anti-PPAR α antibodies. Thus saturated fatty acids likely increase macrophage LPL transcription in a PPARα dependent manner. Nuclear proteins from macrophages incubated with n-3 fatty acids had enhanced binding to the PPRE consensus sequence on the LPL promoter gene and this was not diminished by immunoprecipitation with anti-PPAR α antibodies [20]. The role of PPARs in regulation of arterial wall LPL needs more definition.

ARTERIAL INFLAMMATORY RESPONSE

Inflammatory responses at the level of the arterial wall have a role in many steps of atherosclerosis from monocyte infiltration to lipid accumulation to plaque formation and thrombosis [21]. Inflammatory pathways are now recognized as such a key factor in atherogenesis that inflammatory markers such as CRP are now used to monitor risk of cardiovascular disease [22]. Fatty acids influence many steps in immune responses and inflammatory pathways affecting the arterial wall. Recent studies have evaluated the effect of higher dietary n-3 fatty acid intake on serum levels of CRP, IL-6, soluble E-selectin, soluble intracellular adhesion molecule, and soluble vascular adhesion molecules. All are biomarkers of inflammation and endothelial activation and are associated with increased atherosclerotic progression [23–25]. Subjects with higher dietary n-3 fatty acid intake had decreased serum levels of all inflammatory biomarkers, including a 29% decrease in CRP concentration [26].

Neutrophils, lymphocytes, and monocytes typically have high content of arachidonic acid, but oral administration of EPA and DHA results in proportional increases of n-3 fatty acid levels in these cells [27–29]. Specific fatty acids affect signal transduction pathways, gene expression, lipid mediators, phagocytosis, T-cell function, and antigen presentation [30,31] and these mechanisms likely relate to how saturated and n-3 fatty acids differentially affect arteries. As well, the potent anti-inflammatory and inflammatory resolving properties of resolvins and neuroprotectins may play a significant role in atherosclerotic prevention [32].

Neuroprotectins and resolvins are lipid mediators derived from DHA and EPA and may be a key part to understanding n-3 fatty acids and inflammation in the arterial wall [33]. Resolvin E1 is generated during the resolution phase of inflammation [34]. Of interest, resolvins are produced in human vasculature by the interactions of vascular endothelial cells and leukocytes. They have been shown in vivo to decrease PMN transmigration and block PMN transmigration [35]. Resolvins may play a key role in atherosclerosis prevention, but currently details on effects of resolvins in the arterial wall in vivo are limited [36].

A potential role for resolvins in atherogenesis is suggested by the influence of blood resolvins on the cell adhesion molecules of polymorphonuclear leukocytes and monocytes that are essential to extravasation [37]. Leukocyte recruitment, which is central in atherosclerosis, involves interaction of leukocyte surface adhesion molecules with endothelial cells [38]. Contact with the inflamed endothelium facilitates leukocyte rolling and a transient pause of leukocytes on the endothelium via L-selectin [39]. When blood from human subjects was incubated in vitro with resolvinE1, L-selectin was rapidly shed from both monocytes and PMNs [37]. While L-selectin has a role in the transient interaction of circulating leukocytes, CD18 integrins are involved in the stable adhesion and transmigration of neutrophils [39]. CD 18

integrins bind to the endothelial counter receptors, which are intercellular adhesion molecule 1 and 2 [39,40]. Also, following human blood incubation in vitro with resolvinE1, CD18 expression on PMN and monocytes was significantly reduced [37].

Inflammatory cytokines such as TNF-α activate endothelial cells to express selectin adhesion receptors that form strong transient bonds to the carbohydrate counter ligands on the surfaces of neutrophils [41,42]. This transient neutrophilic adhesion to the vascular endothelium is termed rolling and is the first step in leukocyte migration across the endothelial wall [43]. Thus, fatty acids may have a role in the inflammatory process at the level of the arterial wall through modulating neutrophilic migration across the endothelial barrier of arteries.

Leukocyte rolling and adhesion to the arterial wall contributes to atherogenesis [44,45]. In vivo, resolvins were reported to reduce leukocyte rolling in venules. Resolvin E1 was administered intravenously and intravital microscopy showed a subsequent 40% reduction in leukocyte rolling [37]. Resolvin E1 added to human platelet rich plasma specifically blocked ADP-stimulated platelet aggregation and thromboxane receptor stimulated platelet aggregation [37]. This may be related to the inhibition of platelet aggregation seen with high dose EPA supplementation. Further studies need to be done on the role of resolvins on endothelial function so that their role in arterial wall protection can be further clarified and possibly be used as a target for pharmacotherapy.

Tull et al. reported that the transit of neutrophils across the vascular endothelial cell monolayer was significantly reduced by n-3 fatty acids [46]. Vascular endothelial cells were incubated with EPA and arachidonic acid (AA), which resulted in the incorporation of EPA and AA into cellular phospholipid. The endothelial cells were then stimulated by TNF-α and neutrophils were introduced. Neutrophils migrated across the TNF-α stimulated vascular endothelial cells exposed to AA but did not transmigrate endothelial cells exposed to EPA [46]. This difference may be related to cyclooxygenases (COX) produced by vascular endothelial cells. COX enzymes metabolize AA into prostaglandin D_2 (PGD_2), reported to have a role in neutrophil recruitment and migration in vivo [47,48]. When EPA replaced AA as the substrate for cyclooxygenases prostaglandin D_3 (PGD_3) was generated. The EPA metabolite PGD_3 was reported to block PGD_2 from interacting with the neutrophil PGD_2 receptor and prevented neutrophils from migrating across the endothelial barrier [46]. Another study by Massaro et al. showed that high dose DHA attenuated interleukin-1 (IL-1) induced expression of COX-2 in endothelial cells [49].

Mullen et al. reported that EPA and DHA attenuated inflammatory cytokine production by activated macrophages [50]. Both EPA and DHA decreased macrophage production of inflammatory cytokines IL-1β and IL-6. Only EPA decreased macrophage production of TNF-α. N-3 fatty acids likely modulated cytokine production by significantly reducing a key regulator of cytokine transcription, the cytoplasmic I-kappa-B kinase-beta (IKKβ) complex [50]. Some of the more recently defined pathways of inflammatory resolution seen with n-3 fatty acids and metabolites may represent new preventive and therapeutic approaches.

CELL PROLIFERATION

The proliferation of vascular smooth muscle cells and their lipid accumulation play a significant role in the pathophysiology of atherosclerosis [51]. Several studies have shown how fatty acids influence vascular smooth muscle cells in cell culture [52]. Terano et al. incubated EPA and DHA with vascular smooth muscle cells [53]. EPA and DHA were incorporated into phospholipids, and this was linked to suppressed vascular smooth muscle cell proliferation. EPA and DHA attenuated the progression of cells through the synthesis phases of the cell cycle by inhibiting DNA synthesis and replication. Specifically EPA and DHA inhibited the

phosphorylation of Cdk2 protein and Cdk2 kinase activity, which have roles in cell cycle regulation and proliferation [53].

Bermudez et al. also reported that dietary fatty acids can influence human coronary smooth muscle cell proliferation, a known contributor to atherosclerosis and plaque stability [54]. Postprandial triglyceride rich lipoproteins were prepared from the plasma of human subjects after the consumption of butter and thus had a high concentration of saturated fatty acids. The postprandial triglyceride-rich lipoproteins prepared from human subjects after consumption of vegetable and fish oils were highest in n-3 fatty acids. These triglyceride-rich particles were incubated for 24 hours in a cell culture with human coronary artery smooth muscle cells. Triglyceride-rich particles with highest concentrations of saturated fats enhanced coronary smooth muscle cell proliferation in a dose dependent manner. This proliferation was likely through the activation of genes that forced these smooth muscle cells to enter the synthesis phase of the cell cycle. The triglyceride-rich particles taken after the consumption of fish and vegetable oils attenuated smooth muscle cell proliferation [54].

VULNERABLE PLAQUE

Vulnerable plaques are unstable atherosclerotic lesions at risk of rupturing and leading to arterial obstruction. Some suggest an expanded definition of the vulnerable plaque to also include those at high risk of rapid progression [55]. The early stages of atherosclerosis involve fatty streak formation and macrophage lipid uptake. Over time reactive oxygen species, smooth muscle cell migration, and matrix deposition combine with foam cells to develop into a plaque with a fibrous cap and a lipid core. As the plaque undergoes continued inflammation, matrix degradation, hypoxemia, angiogenesis, and cell death, its fibrous cap thins and its lipid core becomes necrotic. The thinning fibrous cap and enlarging lipid core increase the circumferential stress on the plaque and lead to the transitional point in the plaque's risk of rupture [56]. During this transition, increased monocyte, macrophage, and T-lymphocyte infiltration are also seen. With the thin fibrous cap, large lipid core, and high inflammatory content, there is an increased risk of intraplaque hemorrhage and rupture, i.e. the plaque is "vulnerable." Other histologic characteristics include fissured plaque, intraplaque hemorrhage, and remodeling.

The specific mechanisms by which an advanced plaque becomes a vulnerable plaque are still being defined. Many of the pathways involved in plaque eruption and progression are likely sensitive to modulation by fatty acids. For example, for first time myocardial infarction patients' mortality decreased by 29% after consuming n-3 fatty acid rich fish, twice a week for two years [57]. One randomized double blind placebo controlled trial looked at the effects of n-3 fatty acids on patients with evidence of coronary artery disease on angiogram. After two years of supplementation the angiograms were repeated and showed statistically significant difference in mild and moderate regression in coronary segments between n-3 fatty acid and control groups [58].

In a randomized controlled trial, the influence of fish oil supplementation on plaque stability was evaluated in patients who were awaiting carotid endarterectomy [59]. Patients were given fish oil supplements at current recommended dosages for a median for 42 days. The carotid plaques were then surgically removed and analyzed. N-3 fatty acids were incorporated into the phospholipids, cholesteryl esters, and triacylglycerols of the carotid plaques. Plaque stability was measured by the plaque's macrophage infiltration and morphology. N-3 fatty acid supplementation resulted in decreased macrophage infiltration and a more stable plaque morphology, as per the American Heart Association's lesion classification system [60]. N-3 fatty acid intake was associated with more milder type IV lesions (atheromas), more plaques with thick fibrous caps, and fewer plaques with thin inflamed caps [59].

CONCLUSIONS

The World Health Organization reports that cardiovascular diseases are the number one cause of death globally and that an estimated 23.6 million people will die in 2030 from cardiovascular diseases, mainly heart disease and stroke. Atherosclerosis is the underlying cause of most cardiovascular diseases, including heart failure and stroke. Dyerberg and Bang linked n-3 fatty acids to decreased risk of cardiovascular disease in 1979 [61]. They reported that Inuit Eskimos' high n-3 fatty acid consumption likely prevented cardiovascular disease by decreasing circulating lipids, prolonging bleeding time, and decreasing platelet aggregation [62]. Since then many studies have confirmed the cardioprotective effects of n-3 fatty acids in both primary and secondary prevention trials [2,63]. In this paper we have described a number of pathways whereby n-3 and saturated fatty acids may influence arterial lipid deposition, inflammation, cell proliferation, and plaque vulnerability (Table 1). Overall, data linking the mechanisms of action of n-3 fatty acids to prevention of coronary heart disease in humans is still limited. Resolvins and protectins, potent anti-inflammatory chemical mediators generated from EPA and DHA, may be key to contributing to mechanisms of cardioprotection. It is possible that these new lipid mediators will prove to have beneficial roles in preventing atherosclerosis, plaque instability, and coronary heart disease.

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Table 1

Comparison of the effects of n-3 fatty acids versus saturated fatty acids on pathways involved in atherosclerosis and plaque vulnerability. Relevant references are provided in the text.

Pathway	Effect of n-3 fatty acids	Effect of saturated fatty acids
ARTERIAL LIPID DEPOSITION	↓	↑
• Arterial LPL expression	↓	↑
• Cholesterol delivery	↓	↑
– Total LDL uptake	↓	↑
– Selective uptake of cholesterol	↓	↑
• Cholesterol efflux	↑	↓
ARTERIAL INFLAMMATORY RESPONSE	↓	↑
• Macrophage NFκB activity	↓	↑
• Expression of IL-6	↓	↑
• Circulating C-reactive protein	↓	↑
• Expression of cellular adhesion molecules	↓	↑
• Inflammatory resolution	↑	↓
• Leukocyte recruitment	↓	↑
• Leukocyte rolling & adhesion	↓	↑
• Leukocyte transmigration	↓	↑
CELL PROLIFERATION	↓	↑
• Platelet aggregation	↓	↑
• Vascular smooth muscle cell proliferation	↓	↑
PLAQUE VULNERABILITY	↓	↑
• Plaque macrophage infiltration	↓	↑
• Stable plaque morphology	↑	↓