Omega-3 fatty acids and leukocyte-endothelium adhesion: novel anti-atherosclerotic actions

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Abbreviations used:

CAM, cellular adhesion molecule;

CHD, coronary heart disease;

CRP, C-reactive protein;

CVD, cardiovascular disease;

DHA, docosahexaenoic acid;

DPA, docosapentaenoic acid;

EC, endothelial cell;

EPA, eicosapentaenoic acid;

FA; fatty acid,

FAMEs; fatty acid methyl esters

HAECs, human aortic endothelial cells;

HCAECs, human coronary artery endothelial cells

HIMECs, human intestinal microvascular endothelial cells;

HSaVECs, human saphenous vein endothelial cells;

HUAECs, human umbilical artery endothelial cells;

HUVECs, human umbilical vein endothelial cells;

ICAM-1, intercellular adhesion molecule-1;

IL-6, interleukin-6;

IL-8, interleukin-8;

LDL, low-density lipoprotein;

LFA-1, Lymphocyte function-associated antigen

MCP-1, monocyte chemoattractant protein 1;

NF-kB, nuclear factor kappa-light chain enhancer of activated B cells;

oxLDL, oxidised low-density lipoprotein;

PUFA, polyunsaturated fatty acid;

RANTES, regulated on activation, normal T cell expressed and secreted;

SAA, serum amyloid A;

SMCs, smooth muscle cells

TNFα, tumour necrosis factor alpha;

VEGF, vascular endothelial growth factor;

VCAM-1, vascular cell adhesion molecule-1;

VLC, very long-chain;

n-3, omega 3.

Abstract

Endothelial cells (ECs) play a role in the optimal function of blood vessels. When endothelial function becomes dysregulated, the risk of developing atherosclerosis increases. Specifically, upregulation of adhesion molecule expression on ECs promotes the movement of leukocytes, particularly monocytes, into the vessel wall. Here, monocytes differentiate into macrophages and may become foam cells, contributing to the initiation and progression of an atherosclerotic plaque. The ability of omega-3 (n-3) polyunsaturated fatty acids (PUFAs) to influence the expression of adhesion molecules by ECs and to modulate leukocyte-endothelial adhesion has been studied in cell culture using various types of ECs, in animal feeding studies and in human trials; the latter have tended to evaluate soluble forms of adhesion molecules that circulate in the bloodstream. These studies indicate that n-3 PUFAs (both eicosapentaenoic acid and docosahexaenoic acid) can decrease the expression of key adhesion molecules, such as vascular cell adhesion molecule 1, by ECs and that this results in decreased adhesive interactions between leukocytes and ECs. These findings suggest that n-3 PUFAs may lower leukocyte infiltration into the vascular wall, which could contribute to reduced atherosclerosis and lowered risk of cardiovascular disease.

1. Introduction

Atherosclerosis is an inflammatory disease of the vascular wall, usually within an artery [1-5]. It results from interactions between modified lipoproteins, immune cells and the endothelium [1-6]. The arterial wall becomes thickened because of the accumulation of lipid-loaded macrophages (foam cells), smooth muscle cell proliferation and deposition of extracellular matrix proteins to form a fibrous cap. Infiltration of monocytes and their differentiation to macrophages is one of the earliest events in atherosclerosis; thus, endothelial dysfunction and monocyte-endothelial interactions are key to the initiation and progression of atherosclerosis [1-6].

In normal physiological conditions, the endothelium contributes to vascular homeostasis by active regulation of vascular tone, control of permeability between the bloodstream and the underlying vascular wall, regulation of medial smooth muscle cell growth, and control of platelet function, coagulation and fibrinolysis. Inflammatory stimuli, such as cytokines and bacterial endotoxin lead to endothelial activation, whereby endothelial cells (ECs) undergo functional changes influencing their interactions with blood leukocytes. These changes play an important role in the initiation of adhesion of monocytes to the endothelium, which precedes their infiltration into the vascular wall.

Many studies have indicated that consumption of very long chain (VLC) omega-3 (n-3) polyunsaturated fatty acids (PUFAs) reduces the risk of developing coronary heart disease (CHD) [7, 8]. These findings are largely based on association studies, but are supported by studies of the effects of n-3 PUFAs on risk factors for CHD. In contrast, some recent meta-analyses indicate a limited role for n-3 PUFAs in preventing mortality in patients with existing CHD [9, 10].

There is good evidence that VLC n-3 PUFAs can favourably modify several biological processes which play a role in promoting atherosclerosis [11], including inflammation [12, 13]. These beneficial modifications, which would result in preventing the initiation and slowing the progress of atherosclerosis, include a reduction in endothelium-leukocyte interactions. Indeed, several studies have shown that the two main bioactive n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), lower the expression of adhesion molecules involved in leukocyte-endothelial cell interactions (see later for more details).

The aim of this article is to review studies reporting the effects of n-3 PUFAs (EPA and DHA) on adhesion molecule expression by ECs and on leukocyte-endothelial cell adhesion.

2. Adhesion molecules and the role of leukocyte-endothelium interactions in atherosclerosis

Arteries and veins are tubular vessels comprising of 3 layers; an inner endothelium, a middle intimal layer and an outer *tunica externa*. The outer layer contains connective tissue and the *vasa vasarum*; the middle layer comprises primarily of smooth muscle cells and the inner layer is a monolayer of ECs which are in direct contact with free flowing blood [14].

The endothelial lining of the vasculature is both anti-thrombogenic and anti-adhesive, allowing free flow of blood and its components. However, in an inflammatory state, the endothelium is rendered adhesive for circulating leukocytes, initiating the process of leukocyte recruitment, which involves their rolling, adhesion and migration into the intima [15]. Figure 1 depicts the adhesion, activation and migration of leukocytes. Leukocyte recruitment is controlled by expression of adhesion molecules and chemokines on the surface of the inflamed endothelium. Chemokines activate the circulating cells (i.e. the leukocytes) causing them to bind to the endothelium in order to initiate leukocyte migration across the endothelium. Once in the tissue, the leukocytes migrate towards the site of infection or injury by chemotaxis, the process of chemical attraction along a concentration gradient. Examples of chemoattractants include monocyte chemoattractant protein 1 (MCP-1), regulated on activation, normal T cell expressed and secreted (RANTES), interleukin 8 (IL-8), leukotriene B_4 and certain microbial peptides.

Adhesion molecules expressed on both ECs and leukocytes play an important role in leukocyte-endothelium interactions. The adhesion molecules expressed on ECs are selectins (e.g. E-selectin, P-selectin, L-selectin) and members of the immunoglobulin superfamily (e.g. intercellular adhesion molecule 1, 2 and 3 (ICAM-1, ICAM-2, ICAM-3) and vascular cell adhesion molecule 1 (VCAM-1)). Adhesion molecules expressed on the surface of leukocytes act as counter-receptors for those on ECs and include integrins and selectins (e.g. P-selectin glycoprotein-1 (PSGL-1) which binds to E, L and P-selectin) [16]. Table 1 lists examples of adhesion molecule pairs and some of their characteristics.

The earliest step of leukocyte transmigration involves weak reversible interactions between the endothelial selectins (E-selectin, P-selectin and L-selectin) and their counter ligands, PSGL-1 and E-selectin ligand-1 (ESL-1), causing rolling and tethering of leukocytes along the vascular endothelium [17-20]. Leukocyte slowing during the rolling promotes the attachment of specific high affinity G-protein coupled receptors by EC activating factors, such as chemokines [21]. The β2 and β1 family of integrins are the major integrins that are involved in this stage of the leukocyte/EC interaction. They bind to EC counter ligands, such as ICAM-1 and VCAM-1 [22]. Integrins mediate both the firm adhesion of leukocytes to ECs, as well as the flattening of leukocytes over the endothelium [15, 22]. Once adhered, leukocytes become activated, causing them to de-adhere and migrate through the vessel wall by traversing the EC layer and the basement membrane [23], a process known as diapedesis. A major leukocyte integrin is lymphocyte function-associated antigen (LFA)-1, which binds to ICAM-1 and ICAM-2 and triggers leukocyte searching of endothelial boundaries [24]. The shedding of L-selectin from the leukocyte must occur for this to happen and this is promoted by the release of mediators such as IL-8 [25].

Atherosclerotic lesions or plaques originate at branching points of the vascular, usually the arterial, tree, characterised by low or oscillatory shear stress, which favours passive transport of arterial blood components into the vessel wall [26]. Arterial fatty streaks represent the earliest stage of plaque development [23, 27-29]: these were found to be present in coronary arteries of 50% of humans aged 10-14 years in an autopsy study [27], and are the earliest detectable lesions in hypercholesteraemic animal models of atherosclerosis in different species [23, 29, 30].

Fatty streaks are areas of intimal thickening produced by the accumulation of lipid-laden foam cells, which become surrounded by an extracellular matrix and lymphocytes. These fatty streaks have been shown to precede more advanced atherosclerotic lesions in various animal models; however, their ability to be reversed is also accepted [31, 32].

Low-density lipoproteins (LDLs) trapped in the sub-endothelial layer trigger an inflammatory response [33]. The LDLs which are retained by the intima subsequently undergo oxidative modification to form oxidised LDL (oxLDL) [33]. oxLDL causes a low-grade chronic inflammatory response in the vessel wall, leading to increased expression of cell-adhesion

molecules, chemokines and pro-inflammatory cytokines [3, 33]. Cell adhesion molecules promote further adhesion of blood monocytes to the endothelium and increased secretion of chemokines, including MCP-1, which in turn promotes the migration of these monocytes into the intima, where they undergo differentiation into macrophages [34]. Macrophages in the intima then proceed to engulf oxLDL through scavenger receptors forming the lipid-laden foam cells characteristic of atherosclerotic plaques [34]. The chronic inflammatory response continues as macrophages secrete various cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNFα). These act to enhance endothelial permeability, therefore allowing entry of more LDL and monocytes. As the inflammation advances, macrophages and endothelial cells release a variety of peptide growth factors, which act as fibrogenic mediators. These promote smooth muscle cells to proliferate and migrate from the media to the site of the early lesion. Here they form a dense extracellular matrix that acts as a cap, which covers the accumulating lesions [1].

Increasing levels of IL-1 and TNFα continue to drive the inflammatory response and stimulate the production of IL-6. This sustains the inflammation and induces distal inflammatory responses, including hepatic synthesis of acute phase proteins such as C-reactive protein (CRP) and serum amyloid A (SAA) [14]. Inflammatory processes then act to both increase the activity of matrix metalloproteinases, which degrade the dense extra-cellular matrix of the lesion cap, whilst simultaneously decreasing collagen production, which is stabilising. This leads to lesion weakness and possible cap rupture. Plaque rupture exposes the pro-thrombotic plaque interior to blood and initiates platelet aggregation and clot (thrombus) formation. IL-6 can also contribute to thrombus formation through stimulation of expression of the potent procoagulant tissue factors [1, 35]. Extensive clotting may occlude blood flow to the affected organ initiating a cardiovascular event (e.g. myocardial infarction, stroke) and may be fatal.

Thus, abnormal inflammation caused by endothelial dysfunction and leukocyte infiltration into the blood vessel wall plays a central role in the development and progression of atherosclerosis. Endothelial injury or stimulation increases the adhesion of leukocytes (and platelets) by upregulating expression of adhesion molecules, as well as increasing the permeability of the endothelium. Figure 2 shows the processes involved in atherosclerosis and the interactions between ECs and inflammatory cells.

The concentrations of the soluble adhesion markers sVCAM-1 and sICAM-1 in the bloodstream have been shown to be significantly correlated with carotid intima thickness, an index of early atherosclerosis [36]. sVCAM-1 has also been shown to indicate the severity of atherosclerosis and is significantly correlated to peripheral atherosclerosis [37, 38]. Another study, which evaluated the sICAM-1 and sVCAM-1 levels in peripheral arterial disease (PAD), found that sICAM-1 was a better marker of both the presence and the progression of disease [39]. The Physicians Health Study also indicated that sICAM-1, though not sVCAM-1, was an indicator of development of PAD [40]. Few studies indicate an effect of selectin levels on development of atherosclerosis, although it has been reported that an increase in sE-selectin may predict restenosis after percutaneous transluminal angiography [41]. In other smaller studies increased sP-selectin was associated with PAD [42]. Although these studies describe correlation of adhesion markers and the extent of atherosclerosis, meta-analyses have concluded that soluble adhesion molecules are unlikely to add to preexisting predicitive risk factors and that their prognostic value remains unclear [43, 44].

3. Effects of EPA and DHA on expression of adhesion molecules and leukocyte-endothelium interactions

PUFAs are important constituents of the phospholipids of all cell membranes, where they regulate cell signalling, membrane protein function, membrane fluidity and overall cellular function. Typically, those consuming a Western diet have low amounts of EPA and DHA in the phospholipids of inflammatory cells (neutrophils, lymphocytes, monocytes). This however can be modified by increasing intake of these PUFAs, leading to higher concentrations of EPA and DHA in the cells [45-47].

EPA and DHA have been described to decrease the expression of adhesion molecules on the surface of various EC types (see Table 2), effects that could lead to a decrease in leukocyte infiltration, reduced inflammation, and greater plaque stability. It has been demonstrated that individuals consuming increased amounts of EPA and DHA have a lower risk of cardiovascular events and mortality [7, 8]. This could be explained in some part by a reduction in atherosclerosis. LC n-3 PUFAs have been shown to help to stabilise plaques [48].

3.1 In vitro studies

There is a wide choice of human ECs for in vitro investigations, including human aortic ECs (HAECs), human coronary artery ECs (HCAECs), human intestinal microvascular ECs (HIMECs), human saphenous vein ECs (HSaVECs) and human umbilical artery ECs (HUAECs). Many, although not all, of these cells are of aortic or arterial origin, which is appropriate since atherosclerosis is mainly an arterial disease. However, the most common ECs studied are human umbilical vein ECs (HUVECs). While it may seem odd to use cells of venous origin to model events leading to an arterial disease, HUVECs are an appropriate model for several reasons. Firstly, the umbilical vein carries oxygenated, nutrient-rich blood from the placenta to the fetus, and in this regard is more like an artery than a vein. Secondly, HUVECs respond in a very similar way to arterial and aortic ECs, both qualitatively and quantitatively, to an inflammatory signal (Figure 3). Thirdly, HUVEC monolayers bind leukocytes under both static [49, 50] and flow [50-52] conditions and those leukocytes exhibit diapedesis just as they would through arterial EC monolayers. Fourthly, umbilical veins have been used for grafting into the arteries of human patients; such venous grafts develop atherosclerosis that is the same as seen in arteries [53, 54], indicating that the umbilical vein is not different from the artery in terms of the ability to develop atherosclerosis. Finally, an increase in intima media thickness has been reported in umbilical veins from at risk pregnancies [55, 56], suggesting that the umbilical vein *in situ* can present the earliest stages of atherosclerosis. The *in vitro* studies of fatty acids described below used a range of ECs; because of the similar biology across all of these EC types the studies were considered to be comparable. Studies reported a variety of changes in cell-surface expression, protein levels and mRNA expression depending on EPA and DHA treatment. These studies and their findings are summarised in Table 2.

3.1.1 Adhesion molecule expression by ECs

De Caterina et al. were the first to report the effect of n-3 PUFAs on adhesion molecule expression on ECs [49]. HSaVECs treated with 10 μM DHA for 24-96 hr were stimulated with either TNFα or IL-1α for 6 or 24 hr. DHA significantly decreased VCAM-1 cell surface expression (-30%), mRNA levels (-50%) and protein expression (-53%) in TNFα stimulated cells. DHA also decreased protein expression of ICAM-1 (-53%) and E selectin (-23%) in IL-1α stimulated HSaVECs. [49]. Others reported that DHA (5 or 25 μM) decreased VCAM-1 protein expression in IL-1β stimulated HIMECs [57]. In these studies, cells were pre-treated

with DHA (5 or 25 μM) for 24 hr followed by IL-1β stimulation for 8 or 24 hr. DHA pre-treatment significantly decreased VCAM-1 protein expression in response to IL-1β at 5 μM and at 25 μM (-37%, -50% respectively), but had no effect on ICAM-1 protein expression at either concentration. Yates et al. describe decreased cell surface expression of E-selectin (-55%) in DHA treated HUVECs (5 μM for 24 hr) following TNFα activation. However, there was no effect on mRNA or total cellular levels of E-selectin protein [51]. Those authors argued that down-regulation of surface expression of adhesion molecules was caused by modulation of intercellular transport mechanisms, which present adhesion molecules on the surface of stimulated ECs. They also observed no change in ICAM-1 mRNA levels in DHA-treated HUVECs.

Goua et al. investigated the effect of both EPA and DHA on ICAM-1 and VCAM-1 in TNFα stimulated HUVECs and smooth muscle cells (SMCs) [58]. Cells were pre-incubated with EPA or DHA for 24 hr followed by stimulation with TNFα for 6 hr. The study reported inhibition of cell-surface expression of both ICAM-1 and VCAM-1 by both EPA (-18%, -8% respectively) and DHA (-10%, -15% respectively) at 25 μM. Huang et al. described effects of EPA and DHA on protein and mRNA levels of ICAM-1 and VCAM-1 in LPS-activated HAECs [59]. Cells were incubated with EPA or DHA (100 μM) for 24 hr and then treated with LPS for 12 hr. Both EPA and DHA significantly reduced VCAM-1 total protein (-70%, -80% respectively), cell surface expression (-60%, -70% respectively), and mRNA expression (-70%, -90% respectively). EPA and DHA also significantly reduced ICAM-1 total protein (-50%, -70% respectively), cell surface expression (-40%, -50% respectively), and mRNA expression (-40%, -70%, respectively). Chen et al. pre-treated cultured HCAECs with DHA or EPA (10 or 50 μM) followed by incubation with oxLDL for 24 hr [60]. OxLDL induced upregulation of mRNA expression and protein levels of both P-selectin and ICAM-1, which were decreased by EPA and DHA. EPA decreased mRNA concentrations and protein concentrations of P-selectin at both 10 (-31%, -29%) and 50 μM (-67%, -71%) as well as mRNA concentrations and protein concentrations of ICAM-1 at both 10 (-27%, -22%,) and 50 μM (-67%, -83%). DHA also decreased mRNA concentrations and protein concentrations of P-selectin at both 10 (-44%, -41%) and 50 μM (-63%, -71%) as well as mRNA concentrations and protein ICAM-1 at both 10 (-27%,-33%) and 50 μM (-73%, -83%).

Collie-Duguid and Wahle [61] described lower VCAM-1, ICAM-1 and E-selectin mRNA levels in IL-1β stimulated HUVECs after incubation with 65 μM DHA or EPA, although the effects were not statistically significant. Wang et al. too describe the effect of both EPA and DHA, as well as differential effects of both EPA and DHA. [62]. They examined varying concentrations of DHA and EPA (20 to 160 μM) in TNFα stimulated HAECs. They observed a decrease in VCAM-1 cell-surface expression by DHA at 40-160 μM (-20 to -70%) and EPA at 80-160 μM (-20%) in TNFα stimulated HAECs. DHA at 80 μM also significantly reduced VCAM-1 protein expression (-70%) in the cell lysates of TNFα-treated HAECs; this was not seen in EPA treated cells. DHA at 160 μM decreased cell surface expression of ICAM-1 (-20%), though no decrease was seen in ICAM-1 protein expression, and again no effect was seen in EPA treated cells.

In contrast to several other studies, Mayer et al. observed no change in cell surface levels of ICAM-1, VCAM-1 or E-selectin in HUVECs pre-incubated with EPA and DHA at 10 μM and stimulated with TNFα [63].

Thus, several studies show that pre-treatment with EPA or DHA leads to decreased cell-surface expression and cellular protein levels, as well as the down regulation of mRNA expression, of VCAM-1 and ICAM-1 in a variety of EC types. Fewer studies report the effect of EPA and DHA treatment on cell-surface expression, total protein and mRNA levels of selectins (E-selectin and P-selectin) in ECs. Only one study [60] described the effect on P-selectin: there were significant decreases in both P-selectin mRNA and total protein concentration by HCAECs after EPA and DHA treatment. Yates et al. [51] saw no changes in either mRNA or total amounts of E-selectin on TNFα stimulated HUVEC with exposure to DHA (5 μM), but described significant decreases in cell-surface expression of E-selectin.

Together, the findings from these studies demonstrate a role for EPA and DHA in down-regulating expression of adhesion molecules in ECs, especially VCAM-1 and ICAM-1. Since fewer studies examined changes in selectin levels after treatment with n-3 PUFAs, it is unclear whether these are affected. Through effects on adhesion molecule expression, EPA and DHA may down-regulate leukocyte-endothelial adhesive processes which might slow leukocytic infiltration into the vessel wall and limit atherosclerosis development and progression.

3.1.2 Endothelial-leukocyte adhesion

Static adhesion assays carried out by De Caterina et al. and Wang et al. demonstrated reduced adhesion of both THP-1 monocytes [49, 62] and neutrophils [64] to ECs pre-treated with DHA. De Caterina et al. exposed HSaVECs to DHA (10 μM) for 96 hr followed by IL-4 for 24 hr; they reported a significant decrease in THP-1 adhesion to these cells (-31%) [49]. Wang et al. reported 24 hr exposure of HAECs to DHA (80 μM) followed by stimulation with TNFα inhibited adhesion of THP-1 cells (-45%) [62]. However, they observed no change in THP-1 adhesion after EPA treatment of HAECs [62]. Huang et al. reported an effect of both EPA and DHA on THP-1 adhesion to HAECs [59]. HAECs were incubated with EPA or DHA (100 μM) for 24 hr and then treated with LPS for 12 hr; finally, HAECs were co-cultured with the THP-1 cells for 1 hr. Both DHA and EPA significantly attenuated the adhesion of THP-1 cells to HAECs. Using lipid raft isolation and confocal microscopy, they described EPA and DHA as inhibiting key components in the nuclear factor kappa-light chain enhancer of activated B cells (NF-kB) activation pathway. Chen et al. also showed a significant reduction in monocyte adhesion to ox-LDL stimulated HCAECs after pre-treatment of the latter with either EPA or DHA (10 or 50 μM) [60].

Others investigated the effect of EPA in an *in vitro* adhesion assay under more physiological flow conditions; both monocyte rolling and adhesion to LPS-stimulated HUVEC monolayers were reported [65]. Treatment of HUVECs with EPA (50 μM) for 1 hr significantly inhibited monocyte rolling (approx. – 86%) and adhesion (approx. -99%). These effects were linked to a significant decrease in cell-surface expression of both VCAM-1 and ICAM-1 after EPA treatment in the LPS-stimulated HUVECs, although there was no effect of EPA on E-selectin expression. Similarity Mayer et al. investigated the rolling and adhesion of monocytes to HUVECs under laminar flow conditions *in vitro* [63]. HUVECs were pre-incubated with EPA or DHA (10 μM) for 6 hr followed by stimulation with TNFα for 20 hr. Although, as described earlier, EPA and DHA did not affect cell-surface adhesion molecule expression in this study, both EPA and DHA significantly reduced both monocyte rolling and adhesion of monocytes to TNF-α stimulated HUVECs. EPA incubation decreased both adhesion and rolling (-39%, -48% respectively), but with less potency than DHA (-45%, -62% respectively).

Yates et al. described DHA and EPA as modulating different stages of leukocyte recruitment to ECs [51]. They showed reduced recruitment of neutrophils from flow to TNFα stimulated HUVECs pre-treated with DHA at 0.05, 0.5 and 5 μM. They suggested that DHA reduced recruitment via the inhibition of E-selectin, as described above. EPA was shown to inhibit the migration of adherent neutrophils across the endothelium, but did not reduce the number of neutrophils recruited. Yates et al. described this effect as being due to the metabolism of EPA to prostaglandin D_3 which antagonised prostaglandin D_2 function – necessary for neutrophil transit across the endothelial cell monolayer. Others investigated the effects of EPA alone on rolling and adhesion of neutrophils to HUVECs under flow conditions [66]. EPA pre-treatment of HUVECs later stimulated with TNFα reduced the number of neutrophils undergoing transendothelial migration. Instead, neutrophils were observed to continue rolling across the endothelium, rather than crossing the EC monolayer [66].

The findings from the adhesion assays outlined here suggest possible independent actions of EPA and DHA on the adhesion and migration of leukocytes across the endothelium. The majority of studies show a reduction in the adhesion and rolling of leukocytes after incubation of ECs with EPA or DHA, with possible higher potency of DHA. However, Yates et al. suggest EPA and DHA may have different roles in the adhesion process [51]. They argue that EPA reduces migration itself while DHA reduces the initial recruitment of monocytes from flow. This study suggests that EPA and DHA in combination could have greater potency than either fatty acid alone when tackling the development of atherosclerotic lesions.

3.2 Animal feeding studies

Yoshihara et al. analysed the effect of feeding EPA and DHA on abdominal aortic aneurysm (AAA) in apolipoprotein E-deficient mice [67]. AAA was induced by angiotensin 2 infusion and mice were supplemented with EPA and DHA and the development of AAA lesions and macrophage infiltration in the aorta were analysed. EPA and DHA were administered orally (5% by weight of diet) from weeks 10 to 16. Angiotensin 2 infusions were given daily from weeks 12 to 16. EPA and DHA administration significantly decreased aortic mRNA levels of

VCAM-1 (approx.. -37.5% for EPA and -42% for DHA). There was also suppression of both AAA development and macrophage infiltration after EPA and DHA administration.

Yamada et al. also observed suppression of VCAM-1 in EPA-treated C57BL/6J mice [65]. Mice were supplemented with EPA (5% by weight of diet) for 1 week, followed by LPS administration. The authors examined the effect of EPA on LPS-induced monocyte adhesion to the endothelial surface surrounding the orifice of intercostal arteries of the thoracic aorta. EPA significantly inhibited monocyte adhesion, compared to the control group (approx. -66%), as well as endothelial expression of VCAM-1 (approx. -68%).

A rat model of chemically-induced colitis demonstrated that gavage with fish oil (DHA:EPA 1:1) for 14 days resulted in a significant decrease in endothelial VCAM-1 (-40%) [57].

Thus, the few studies that evaluated the effects of dietary EPA and DHA on adhesion molecules in animals all reported decreased expression of VCAM-1.

3.3 Human studies

A number of studies have investigated whether supplementation with VLC n-3 PUFAs for a period of time alters the concentrations of soluble adhesion molecules in the bloodstream. These studies are summarised in Table 3. It should be noted that the exact origin of soluble adhesion molecules is unknown, since some of these adhesion molecules are shared by the endothelium and leukocytes (Table 1). Nor is it clear whether a high soluble adhesion molecule concentration reflects high cellular expression.

Some studies report a reduction in sICAM-1 concentration after increased n-3 PUFA intake. Yusof et al. observed that supplementation with 1.8 g EPA + 0.3 g DHA/d for 8 weeks in healthy middle-aged males resulted in lower sICAM-1 concentrations compared to a placebo group (-9.5%) [68]. They demonstrated that the change in plasma sICAM-1 concentration was significantly inversely related to change in DHA in plasma phosphatidylcholine (as a marker of DHA status), but less so to change in EPA. However, no effect of n-3 PUFAs was observed on other markers measured (sVCAM-1, sE-selectin, and sP-selectin). Eschen et al. investigated supplementation of 2 g or 6.6 g/d of EPA+DHA (1:1 ratio) for 12 weeks in healthy subjects [69]. They also observed a significant decrease in sICAM-1 levels (10.3%) in women consuming 2 g/d of EPA+DHA compared to baseline, as

well as significantly decreased serum sP-selectin levels (-9.9%) in both men and women consuming 6.6 g/d EPA+DHA. However, women consuming the higher 6.6 g/d dose had a significant increase in sVCAM-1 (+6.7%) levels when compared to baseline.

Conversely, Thies et al. observed no changes in sICAM-1 after fish oil supplementation, but they did see decreases in all other markers examined. They investigated the effect of 12 weeks daily supplementation of fish oil (providing 720 mg EPA + 280 mg DHA daily) or DHA alone (~700 mg/d) compared to placebo oil or various oil mixes (Table 3) in healthy older subjects (55-75 yr, n=46) [70]. They observed a significant decrease in plasma sVCAM-1 (-28%) after fish oil supplementation. They also describe a decrease in sE-selectin (-17%) after fish oil supplementation, although this was not significant. They suggested that the effect of the fish oil was due to EPA since DHA alone had no influence on any of the markers examined. Plat et al. reported no change in sICAM-1 or sE-selectin levels; they investigated the effect of 1.1 g/d EPA +DHA, compared to control (oleic acid), for 6 weeks in 11 normolipidemic healthy moderately obese men [71].

Some other studies report increases in adhesion markers after intervention. Paulo et al. investigated the effects of an 8 week dietary intervention in healthy 20-40 yr old male and female subjects [72]. Subjects consuming a fatty fish diet (3 x 150 g salmon/week providing the equivalent of 1.3 g DHA and 700 mg EPA/d) or fish oil (430 mg DHA/d + 633 mg EPA/d) had increased sVCAM-1 levels (+16.1% and +21.9%, respectively), compared to baseline. More recently, a study by Rundblad et al. reported a significant increase in sICAM-1 (+6%) after 8 weeks intervention in those consuming the fatty fish diet (providing 1.37 g EPA, 333 mg DPA and 240 mg DHA/week), with no changes in sVCAM-1 levels compared to baseline [73, 74].

Cazzola et al. examined effects of different doses of EPA in healthy young (18-42 yr) and older (53-70 yr) male subjects [75]. Subjects were randomised to 1.35, 2.7 or 4.05 g EPA/day for 12 weeks. The authors observed no significant changes in sICAM-1 or sVCAM-1 levels in either young or older subjects at any dose of EPA. However, supplementation of the highest dose of EPA (4.05 g/day) significantly increased sE-selectin in the young participants (+22.5%), with no change being observed at any other dose of EPA or in the older subjects.

Other studies have investigated the effect of n-3 PUFA supplementation on soluble adhesion molecules in subjects with pre-existing medical conditions. Omacor supplementation at 4 g/d (EPA as ethyl ester 465 mg/g + DHA as ethyl ester 375 mg/g) in hypertriglyceridaemic subjects for 7 months decreased plasma concentrations of sICAM-1 (-9%), and sE-selectin (-16%), but did not affect sVCAM-1 [76]. Hjerkin et al. looked at the influence of long-term (36 months) intervention with EPA and DHA in 563 men with hyperlipidemia [77]. Subjects who received n-3 PUFA supplementation (2.4g/d EPA +DHA), with or without counselling, had significant decreases in serum sICAM-1 concentrations (-6.4%, -8.4%, respectively). However, there were no changes in sVCAM-1 or sE-selectin levels. Similarly Kelley et al. saw no change in circulating concentrations of sICAM-1, sE-selectin or sVCAM-1 in hypertriglyceridemic men supplemented with DHA (3 g/d for 90 d) [78]. Seljeflot et al. described significant increases in both sVCAM-1 (+8.2%) and sE-selectin (+21.9%) after 6 weeks of n-3 PUFA supplementation (4.8 g/d EPA + DHA) in men with hyperlipidaemia, but there was no change in sP-selectin levels [79]. Similarly Johansen et al. investigated the effect of n-3 PUFA supplementation on 54 male and female patients with advanced coronary disease who had been successfully treated with percutaneous transluminal coronary angioplasty within the previous 6 months [80]. Subjects received a daily supplementation of 2.7 g EPA and 1.6 g DHA for 4 weeks. This increased levels of sVCAM-1 (+24.9%) and sE-selectin (+20%) compared to baseline, although levels of sP-selectin did not change.

Berstad et al investigated the effect of 2.4 g/d EPA+DHA in 171 elderly male subjects (65 to 75 years old) with high risk of CHD [81]. Participants were divided into four intervention groups; n-3 PUFA supplementation (EPA and DHA 2.4 g/d (2:1 EPA:DHA)), n-3 PUFA with dietary advice, dietary advice alone or placebo capsules (corn oil). There was no change in serum concentrations of sVCAM-1, sICAM-1, or sE-selectin after 18 months in any of the groups receiving EPA and DHA compared to baseline. Another study looked at the effects of fish oil intervention on soluble adhesion markers (sICAM-1 and sE-selectin) in 300 myocardial infarction survivors [82]. Patients were divided into 2 groups: those below 65 yr of age and those 65 yr old and above. Subjects were supplemented with 3.4 g EPA and 3.5 g DHA daily or control (corn oil) for 12 months. The authors observed no changes in any of the soluble adhesion markers measured after supplementation, when compared to baseline.

Lindqvist et al. analysed plasma phospholipid EPA and DHA in relation to atherosclerosis in 61 yr old men [83]. A total of 487 men were assessed, 345 were deemed to be healthy and 168 had risk of CVD. They concluded that plasma phospholipid EPA, but not DHA, was inversely associated with carotid and femoral IMT as well as several serum endothelial markers (sICAM-1, sVCAM-1, sE-selectin and sP-selectin) supporting the concept of an effect of EPA on vascular structure and endothelial inflammation. There was no association between plasma EPA and DHA and plaque occurrence in the carotid and femoral arteries.

Moeinzadeh et al. looked at the effects of 6 months n-3 PUFA supplementation (540 mg EPA + 360 mg DHA/d) on sICAM-1 and sVCAM-1 levels in 52 haemodialysis patients. They observed reduced sVCAM-1 levels (-32%), but no change in sICAM-1 [84].

A study in type 2 diabetic subjects who consumed 1.8 g/d EPA for 4 weeks showed a significant decrease in plasma concentrations of sE-selectin (-25%), along with a decrease in sP-selectin (-14%) although this was not significant [85]. Sampson et al. looked at the effects of supplementation of 1.2 g EPA and 0.8 g DHA daily on plasma soluble adhesion markers in both healthy and type 2 diabetic male subjects for 3 weeks [86]. There were no changes in any of the adhesion markers analysed, sICAM-1, sVCAM-1 or sE-selectin.

A more recent study carried out in children, gave 107 healthy 8-14 yr olds either milk enriched with n-3 PUFAs (60 mg EPA +120 mg DHA/d) or non-supplemented whole milk, for 5 months [87]. There was a significant decrease in serum levels of both sE-selectin (-17%) and sICAM-1 (-13.5%) in the group receiving milk with EPA and DHA added. After sex analysis, it was shown that boys, but not girls, also had a significant decrease sVCAM-1 levels (-13.9%).

Another study looked at the effects of a fatty fish diet in pregnant women [88]. 123 pregnant women were either to continue their habitual diet or given 2 x 150 g salmon portions a week (3.45 g/week EPA+DHA) from 20 weeks of gestation until delivery. The authors described an increase in sVCAM-1, sICAM-1 and sE-selectin concentrations as pregnancy progressed. Although plasma sICAM-1 was greater in the control group compared to the fish diet group at week 38, no group vs time interaction was observed, and the fatty fish diet had no overall effect on the parameters measured.

Of those studies which examined the effects of EPA and DHA in subjects with pre-existing conditions, the majority reported no significant changes in plasma or serum levels of sICAM-1, sVCAM-1, sE-selectin or sP-selectin; only one study [77] reported significant decreases in serum sICAM-1 concentrations after fish oil supplementation in subjects with hyperlipidaemia. This may be due to the long supplementation period of 152 weeks. However, that study found no changes in serum sVCAM-1 or sE-selectin levels. Some older studies reported significant increases in serum or plasma sVCAM-1 and sE-selectin levels in subjects with hyperlipidaemia and CHD given n-3 PUFAs [79, 80].

Several studies in healthy male and female subjects saw no changes in plasma or serum levels of sICAM-1, sVCAM-1, sE-selectin or sP-selectin with n-3 PUFAs, although some did report reductions. Of those studies which reported sICAM-1 levels after n-3 PUFA supplementation in healthy subjects, the majority reported no changes [69, 71, 73, 75, 86, 88]. However some studies did see significant decreases [68, 70] with only one study reporting a significant increase in serum sICAM-1 levels after supplementation [73]. There are also mixed reports from those studies which examined sE-selectin levels after supplementation: the majority of studies reported no changes [68, 75, 86, 88], although some reported significant decreases in healthy adults and children [70, 87], whilst one study described an increase [75]. Lastly all but one study reported no change in sVCAM-1 levels after n-3 PUFA supplementation [68-70, 73, 75, 86-88]. Only Paulo et al. saw a significant increase in sVCAM-1 levels in males and females after both dietary intervention and n-3 PUFA supplementation [72].

Thus, the human studies reporting on soluble adhesion molecules show a variety of outcomes after supplementation with n-3 PUFAs. Most of the studies which observed decreases in sICAM-1 and sE-selectin levels used preparations of n-3 PUFAs in which EPA dominated over DHA, with only one study reporting decreases after DHA treatment. This is supported by Lindqvist et al. who reported plasma phospholipid EPA, but not DHA, was inversely associated with sICAM-1, sVCAM-1, sE-selectin and sP-selectin [83]. Levels of sVCAM-1 tend to either show no change after n-3 PUFA supplementation or in some instances increase, particularly in women.

4. Summary, discussion and conclusions

N-3 PUFAs have been suggested to alter adhesion molecule expression , which could contribute to their role in lowering risk of developing heart disease [7]. Studies employing cell culture with various types of EC, animal feeding studies and human trials have investigated the impact of n-3 PUFAs on adhesion molecule expression, leukocyte-endothelial adhesion and levels of soluble adhesion molecules.

In vitro studies investigating the effect of treatment of a variety of ECs with EPA and/or DHA most frequently report decreases in the expression of adhesion molecules, assessed either as mRNA, protein or cell surface expression (summarised in Table 2). Those that looked at the adhesion of monocytes or other leukocytes to ECs most often observed decreased adhesion and transmigration, but with EPA and DHA perhaps having differential effects. The findings from the *in vitro* studies are fairly consistent, but any inconsistencies most likely reflect the concentration of the n-3 PUFAs used, the duration of exposure and the nature of the stimulus used to elicit adhesion molecule expression. It is also important to note that effects of n-3 PUFAs are seen with a variety of ECs of arterial and venous origin. Of the few studies describing feeding studies in mice and rats, all reported reduced VCAM-1 expression after feeding with EPA or DHA or combinations of the two. Thus, pre-clinical studies strongly indicate that EPA and DHA can decrease expression of key adhesion molecules on ECs and that this is linked to reduced monocyte and leukocyte adhesion to ECs under either static or flow conditions. Findings from studies in humans evaluating soluble forms of adhesion molecules in plasma or serum are less clear (summarised in Table 3). Some of these studies do report that n-3 PUFAs reduce levels of soluble adhesion markers (sICAM-1 and sE-selectin), although many studies reported no change and some report increases. The studies involving subjects with pre-existing conditions tend to report a lowering of sICAM-1, sE-selectin and sP-selectin concentrations. Some of these studies suggest a role for EPA rather than DHA. However, the findings from studies of soluble adhesion molecules are difficult to interpret because the exact cellular origin of these molecules is unclear and how circulating levels relate to cell surface expression is not well documented.

Together these studies, especially the in vitro and animal studies, suggest that EPA and DHA act to decrease adhesion molecule expression on ECs and as a result of this reduce key leukocyte (especially monocyte)-endothelial interactions. This would lead to slower and reduced development of atherosclerosis, likely contributing to the clearly established

protection against CHD that is seen with higher intake and blood and tissue levels of EPA and DHA [7, 8]. EPA and DHA seem to show different potency towards adhesion molecule expression on ECs and may act to modulate these through different mechanisms. Any differences between EPA and DHA and the mechanisms underpinning such differences require better elucidation.

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Figure captions

Figure 1. Adhesion, activation and migration of monocytes across the endothelium. Monocytes begin to roll and attach to stimulated endothelial cells expressing cell adhesion molecules including P-selectin, E-selectin, VCAM-1 and ICAM-1. Adherent monocytes migrate into the sub-endothelial space and differentiate into macrophages. Uptake of oxLDL via scavenger receptors leads to the formation of foam cells.

Figure 2. Initiating events leading to development of an atherosclerotic plaque. Foam cells interact with Th1 and Th2 lymphocytes leading to chronic inflammation and secretion of various cytokines, including IL-6, TNFα, RANTES and MCP-1. These inflammatory molecules exert both pro- and anti-atherogenic effects on each of the cellular elements of the vessel wall. Stimulated smooth muscle cells begin to migrate from the medial portion of the arterial wall, proliferate and secrete extracellular matrix proteins which then form a fibrous cap.

Figure 3. LPS induced ICAM-1 and VCAM-1 expression in various endothelial cells in culture. Endothelial cells of different origin were cultured at a concentration of 5×10^4 cells/mL in 200 µL culture medium in 96 well flat-bottom plates to confluence. They were then stimulated with 1 µg/mL bacterial lipopolysaccharide for different durations (6, 12, 24 and 48 hours). The cellular concentrations of ICAM-1 and VCAM-1 were measured by ELISA. Cells were incubated with biotinylated anti-human ICAM-1 or VCAM-1 for 2 hours, followed by incubation with streptavidin horseradish peroxidase for 30 min and substrate solution (1:1 mixture of hydrogen peroxide and tetramethylbenzidine) for 30 min. Development of colour was stopped by the addition of 1 M sulfuric acid and absorbance was determined on a microplate reader at 450 nm. Data are mean of 3 observations and are not previously published.

Table1. Adhesion molecule pairs and their distribution between the endothelium and leukocytes

Adhesion molecule	Other names	Function	Ligand	Distribution		Soluble form
				Endothelium	Leukocytes	
Selectins/ligands						
E-selectin	CD62E, ELAM-1	Capturing and rolling of leukocytes, integrin activation	PSGL-1, CD44 and ESL-1	+		+
E-selectin ligand 1	ESL-1	Capturing and rolling of leukocytes	E-selectin	+	+	
L-selectin	CD62L	Capturing and rolling of leukocytes	Lewis X, CD34, PSGL-1, GlyCAM		+	+
P-selectin	CD62P, GMP140	Capturing and rolling of leukocytes, integrin activation	PSGL-1, Lewis X, CD24	+		+
P-selectin ligand 1	CD162, PSGL-1	Rolling/tethering of leukocytes	E, L and P-selectin		+	+
Immunoglobulins						
ICAM-1	CD54	Rolling, adhesion and crawling of leukocytes, and triggering VE-cadherin phosphorylation	Integrins aLB2, aMB2, aXB2	+	+	+
ICAM-2	CD102	Crawling of leukocytes and initiation of diapedesis	Integrins aLB2, aMB2	+	+	+
ICAM-3	CD50	Firm adhesion of leukocytes	Integrins aLB2, aMB2	+	+	+
JAM-A	-	Leukocyte diapedesis	Integrin aLB2	+	+	
JAM-B	-	Maintenance of endothelial tight junctions	Integrin a4B1, JAM-C		+	
JAM-C	-	Prevents reverse transmigration of leukocytes	JAM-B, Integrin aMB2	+	+	
PECAM-1	CD31	Triggering lateral border recycling	Integrins a4B1,	+	+	+

		compartment in endothelial cells; supporting disconnection of leukocytes from endothelial cells and passage through the basement membrane	a4B7, aDB2			
VCAM-1	CD106	Rolling, adhesion and crawling of leukocytes, and triggering VE-cadherin phosphorylation	PECAM-1, Integrin aVB3	+		+
Integrins						
Integrin a2B1	CD49/CD29, VLA-2	Platelet receptor	Collagen, Laminin			
Integrin a4B1	VLA-4	Firm adhesion of leukocytes	VCAM-1, Fibronectin	+		
Integrin aLB2	CD11a/CD18, LFA-1	Firm adhesion of leukocytes	ICAMs		+	
Integrin aMB2	CD11b/CD18, Mac1, CR3	Firm adhesion of leukocytes	ICAM-1, Fibrinogen, Fibronectin, iC3b		+	
Integrin aXB2	CD11c/CD18	Firm adhesion of leukocytes	ICAM-1, Fibrinogen, iC3b, CD23		+	
Integrin aDB2	CD11d/CD18	Firm adhesion of leukocytes	ICAM-3, VCAM-1		+	
Integrin aVB3	CD51/CD61, VNR	Proliferation, migration of SMCs	PECAM-1, Vitronectin, Fibrin, Fibrinogen, vWF	+		
Integrin aVB5	VNR	Proliferation, migration of SMCs	Vitronectin	+		

*List of adhesion receptors is not exhaustive but represents the current most studied. CR, complement receptor; ELAM, endothelial leukocyte adhesion molecule; ESL-1, E-selectin ligand-1; GlyCAM, glycosylation-dependent cell adhesion molecule; GMP, granule membrane protein; iC3b, inactive product of the complement cleavage fragment C3b; ICAM, intercellular adhesion molecule; JAM, junctional adhesion molecule; LFA, leukocyte functional antigen; Mac, macrophage adhesion ligand; PECAM, platelet endothelial cell adhesion molecule; PSGL-1, P-selectin

glycoprotein ligand 1; SMCs, smooth muscle cells; VCAM, vascular cell adhesion molecule; VE, vascular endothelium; VLA, very late antigen; VNR, vitronectin receptor; vWF, von Willebrand factor

Table 2. Studies investigating the effect of increased EPA and or DHA on adhesion markers in various endothelial cells. The magnitude of statistically significant effects is indicated; where there was no statistically significant effect "None" is entered.

Cell type	**EPA or DHA concentration**	**Stimulus used**	**Outcomes measured**	**Effect of EPA (approx. % change where significant)**	**Effect of DHA (approx. % change where significant)**	**Reference**
HSaVECs	10 µM DHA	TNFα or IL-1α	Cell surface expression			De Caterina et al. (1994)[49]
			VCAM-1	None	-30%	
			Protein			
			VCAM-1	NA	-23%	
			ICAM-1	NA	-53%	
			E-selectin	NA	-52%	
			mRNA			
			VCAM-1	NA	-50%	
HUVECs	65 µM EPA or DHA	IL-1β	mRNA			Collie-Duguid and Wahle (1996)[61]
			VCAM-1	None	None	
			ICAM-1	None	None	
			E-selectin	None	None	
HUVECs	10 µM EPA or DHA	TNFα 20 hr	Cell surface expression			Mayer et al. (2002)[63]
			VCAM-1	None	None	
			ICAM-1	None	None	
			E-selectin	None	None	

HCAECs	10 or 50 μM EPA or DHA	Ox-LDL	Protein ICAM-1 P-selectin mRNA ICAM-1 P-selectin	 -22%, -83% (10, 50 μM) -29%, -71% (10, 50 μM) -27%, -67% (10, 50 μM) -31%, -67% (10, 50 μM)	 -33%, -83% (10, 50 μM) -41%, -71% (10, 50 μM) -27%, -73% (10, 50 μM) -44%, -63% (10, 50 μM)	Chen et al. (2003)[60]
HUVECs	25 μM EPA or DHA	TNFα 6 hr or 24 hr	Cell surface expression VCAM-1 ICAM-1	 -8% -18%	 -15% -10%	Goua et al. (2008)[58]
HIMECs	5 or 25 μM DHA	IL-1β 8 or 24 hr	Protein VCAM-1 ICAM-1	 NA NA	 -37.5%, -50% (5, 25 μM) None	Ibrahim et al. (2011)[57]
HAECs	20-160 μM EPA or DHA	TNFα	Cell surface expression VCAM-1 ICAM-1 Protein VCAM-1 ICAM-1	 -20% (80-160 μM) None None None	 -20, -55,-70% (40-160 μM) -20% (160 μM) -70% (80 μM) None	Wang et al. (2011)[62]

HUVECs	5 µM DHA	TNFα	Cell surface expression			Yates et al. (2011)[51]
			E-selectin	NA	-55%	
			Protein			
			E-selectin	NA	None	
			mRNA			
			E-selectin	NA	None	
			ICAM-1	NA	None	
HAECs	100 µM EPA or DHA	LPS	Cell surface expression			Huang et al. (2015)[59]
			VCAM-1	-60%	-70%	
			ICAM-1	-40%	-50%	
			Protein			
			VCAM-1	-70%	-80%	
			ICAM-1	-50%	-70%	
			mRNA			
			VCAM-1	-70%	-90%	
			ICAM-1	-40%	-70%	

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HAECs, human aortic endothelial cells; HCAECs, human coronary aortic endothelial cells; HIMECs, human intestinal microvascular endothelial cells; HUVECs, human umbilical vein endothelial cells; HSVECs, human saphenous vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; IL-1α, interleukin 1 alpha; IL-1β, interleukin 1 beta; LPS, lipopolysaccharide; NA, not assessed; Ox-LDL, oxidised low-density lipoprotein; TNFα, tumour necrosis factor alpha; VCAM-1, vascular cell adhesion protein 1.

Table 3. Studies investigating the effects of increased EPA and DHA consumption on adhesion markers in adult human subjects.

Subjects	**EPA or DHA form**	**EPA+DHA intake**	**Duration (Weeks)**	**Outcomes investigated**	**Effect of EPA +/or DHA (% change where significant; None where these is no significant effect)**	**Reference**
M with hyperlipidaemia	Oil capsule	4.8 g/d (EPA + DHA)	6	Plasma concentrations of sVCAM-1 sE-selectin sP-selectin	 +8.2% +21.9% None	Seljeflot et al. (1998)[79]
M+F with CHD	Oil capsules	2.7 g/d EPA + 1.6 g/d DHA	4	Serum concentrations of sVCAM-1 sE-selectin sP-selectin	 +24.9% +20% None	Johansen et al. (1999)[80]
M with Type 2 diabetes	Fish oil capsules	1.2 g/d EPA + 0.8 g/d DHA	3	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Sampson et al. (2001)[86]
M	Fish oil capsules	1.2 g/d EPA + 0.8 g/d DHA	3	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Sampson et al. (2001)[73]

M+F	Algal oil capsules	700 mg/d DHA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Thies et al. (2001)[70]
M+F	Fish oil capsules	720 mg/d EPA + 280 mg/d DHA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 -28% None None	Thies et al. (2001)[70]
M aged 65-75 years with risk of CHD	Fish oil capsules	1.6 g/d EPA + 0.8 g/d DHA	78	Serum concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Berstad et al. (2003)[81]
M+F myocardial infarction survivors aged > 65 years	Ethyl ester capsules	3.4 g/d EPA + 3.5 g/d DHA	52	Serum concentrations of sICAM-1 sE-selectin	 None None	Grundt et al. (2003)[82]
M+F myocardial infarction survivors aged < 65 years	Ethyl ester capsules	3.4 g/d EPA + 3.5 g/d DHA	52	Serum concentrations of sICAM-1 sE-selectin	 None None	Grundt et al. (2003)[82]
M+F	Fish oil capsules	0.9 g/d EPA + 0.8 g/d DHA	12	Serum concentrations of sVAM-1 sICAM-1 sP-selectin	 None None None	Eschen et al. (2004)[69]
M+F	Fish oil capsules	3 g/d EPA + 2.9 g/d DHA	12	Serum concentrations of sVCAM-1 sICAM-1 sP-selectin	 None None -9.9%	Eschen et al. (2004)[69]

M with hyperlipidaemia	Fish oil capsules	2.4 g /d EPA+DHA	156	Serum concentrations of sVCAM-1 sICAM-1 sE-selectin	 None -6.4% None	Hjerkinn et al. (2005)[77]
	Fish oil capsules + Dietary counselling	2.4 g /d EPA+DHA	156	Serum concentrations of sVCAM-1 sICAM-1 sE-selectin	 None -8.4% None	
M aged 18-42 yr	EPA-rich oil capsules	1.35 g/d EPA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Cazzola et al. (2007)[75]
M aged 18-42 yr	EPA-rich oil capsules	2.7 g/d EPA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Cazzola et al. (2007)[75]
M aged 18-42 yr	EPA-rich oil capsules	4.05 g/d EPA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None +22.5%	Cazzola et al. (2007)[75]
M aged 53-70 yr	EPA-rich oil capsules	1.35 g/d EPA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Cazzola et al. (2007)[75]

M aged 53-70 yr	EPA-rich oil capsules	2.7 g/d EPA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Cazzola et al. (2007)[75]
M aged 53-70 yr	EPA-rich oil capsules	4.05 g/d EPA	12	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Cazzola et al. (2007)[75]
M	Fish oil capsules	0.6 g/d EPA + 0.5 g/d DHA	6	Plasma concentrations of sICAM-1	 None	Plat et al. (2007)[71]
M+F	Fatty fish (2 x 150 g salmon/week) Fish oil capsules	2 g/d (0.7 EPA + 1.3 DHA) 0.63 g/d EPA + 0.43 g/d DHA	8	Serum concentrations of sVCAM-1 Serum concentrations of sVCAM-1	 +16.1% +21.9%	Paulo et al. (2008)[72]
M	Fish oil capsules	1.8 g/d EPA + 0.3 g/d DHA	8	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin sP-selectin	 None -9.5% None None	Yusof et al. (2008)[68]
M+F children	Milk enriched with EPA + DHA	60 mg/d EPA + 120 mg/d DHA	20	Serum concentrations of sVCAM-1 sICAM-1 sE-selectin	 None -13.5% -17%	Romeo et al. (2011)[87]

F (pregnant)	2 x 150 g salmon/week	3.45 g /wk (1.14 EPA + 2.32 DHA)	From 20 weeks of gestation until delivery	Plasma concentrations of sVCAM-1 sICAM-1 sE-selectin	 None None None	Garcia Rodriguez et al. (2012)[88]
M+F	Fish oil capsules	3.1 g/wk EPA + 1.5 g/wk DHA	8	Serum concentrations of sVCAM-1 sICAM-1	 None None	Rundblad et al. (2018)[73]
M+F	Diet intervention lean + fatty fish	4.1 g/wk (1.37 g EPA, 333 mg DPA and 2.40 g DHA)	8	Serum concentrations of sVCAM-1 sICAM-1	 None +6.5%	Rundblad et al. (2018)[73]