Abdominal aortic aneurysm and omega-3 polyunsaturated fatty acids: mechanisms, animal models, and potential treatment

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Abbreviations: Abdominal aortic aneurysm, AAA; Long chain omega-3 polyunsaturated fatty acids, LC n-3 PUFAs; reactive oxygen species, ROS; Toll-like receptor 4, TLR4

Abstract

Abdominal aortic aneurysm (AAA) is an inflammatory disease associated with macrophage accumulation in the adventitia, oxidative stress, medial elastin degradation and aortic dilation. Progression of AAA is linked to increased risk of rupture, which carries a high mortality rate. Drug therapies trialled to date lack efficacy and although aneurysm repair is available for patients with large aneurysm, peri-surgical morbidity and mortality have been widely reported. Recent studies using rodent models of AAA suggest that long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) and their metabolites can moderate inflammation and oxidative stress perpetuated by infiltrating macrophages and intervene in the destruction of medial elastin. This review examines evidence from these animal studies and related reports of inhibition of inflammation and arrest of aneurysm development following prophylactic supplementation with LC n-3 PUFAs. The efficacy of LC n-3 PUFAs for management of existing aneurysm is unclear and further investigations involving human clinical trials are warranted.

Key words: Abdominal aortic aneurysm; Long chain omega-3 polyunsaturated fatty acids; Inflammation; Oxidative stress

1. Introduction

Abdominal aortic aneurysm (AAA) is characterised by a complex and multifactorial pathology involving a degenerative process of segmental weakening and dilation that is typically asymptomatic until rupture [1]. A diagnosis of AAA is conferred by a finding of aberrant enlargement of the abdominal aortic wall beyond 3 cm or expansion in excess of 50% the normal vessel diameter [2]. AAA is classified according to its location relative to the renal artery origins. Most are fusiform, with approximately 80-85% infrarenal, 2% suprarenal and the remainder pararenal [3, 4]. AAA is associated with being male, atherosclerosis, hypertension, cigarette smoking and a genetic predisposition [5]. In a screening program of 700,000 men aged 65 years, 1.34% had an AAA [6]. To date, no drug therapies exist to diminish AAA expansion and current management involves optimisation of cardiovascular health and prolonged intermittent imaging with the option of elective aneurysm repair when rupture risk exceeds that of peri-surgical morbidity and mortality [7]. Several recent studies have reported anti-inflammatory and antioxidant benefits of long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) in animal models of AAA with evidence for amelioration of elastin degradation and aneurysm development. This review discusses mechanisms by which LC n-3 PUFAs intervene in inflammatory and oxidative stress signalling and highlights evidence from animal studies indicating their therapeutic potential in AAA.

2. Abdominal aortic aneurysm: pathogenesis

The histopathologic appearance of AAA, despite considerable variation, typically includes a transmural inflammatory response and extracellular matrix degeneration characterised by disruption and destruction of the medial elastic lamellae and altered collagen microarchitecture [8, 9]. Preclinical evidence from rodent models of AAA suggests that treatment strategies aimed at ameliorating vascular inflammation and proteolytic activity may reduce AAA growth by correcting impaired molecular and mechanical signalling mechanisms in the aortic wall [10].

Aneurysmal degeneration is a complex pathological process involving immune-inflammatory responses and destructive remodelling of aortic connective tissue and progressive dilation affecting all layers of the vascular wall [11, 12]. The inflammatory properties of AAA are characterised by an infiltrate of macrophages, B- and T-lymphocytes, neutrophils and mast cells that are associated with activity of a variety of cytokines (interleukin-1β, IL-6, IL-8, tumour necrosis factor-α and interferon-γ), and local and systemic effects that support further inflammatory cell recruitment and proliferation, promote neo-angiogenesis and enhance matrix turnover [13, 14]. Matrix turnover occurs through cytokine-mediated activation of pathways leading to the subsequent activation of constitutive and inducible proteases (matrix metalloproteinases, cathepsins and serine proteases) [15]. These molecular mediators, derived from intrinsic vessel wall components (medial smooth muscle cells and adventitial fibroblasts) or the cells of the lymphomonocytic infiltrate, are part of an accelerated proteolytic cascade that is responsible for progressive destruction of structural matrix proteins, particularly collagen and elastin, leading to a thin degraded media with significant loss of elastic component and a fibrotic and/or inflammatory adventitia [16, 17]. In addition, cytokines activate apoptotic cell death pathways to reduce smooth muscle cell number and exacerbate elastic media tissue injury [18, 19]. At the macroscopic level, clinically relevant AAA formations commonly include a haemoglobin-rich, non-occlusive intraluminal thrombus (ILT) consisting of a layered fibrin clot underlying haematic luminal and fibrinolysed abluminal layers [20]. The luminal endothelial layer undergoes continuous renewal and is a significant source of pro-inflammatory factors and reactive oxygen species (ROS) that enhance inflammation and intensify proteolytic activity in the aortic wall to result in accelerated AAA growth and a heightened rupture risk [21].

3. Current drug therapies for AAA lack efficacy for retardation of aneurysm growth

Management of small AAA (30-54 mm) relies on optimising the cardiovascular health of the patient and regular surveillance of aortic diameter to determine aneurysm growth [22, 23]. Prompt surgical intervention is recommended for cases meeting predefined criteria that include AAA >55 mm in men,

or >50 mm in women, or enlargement >10 mm/year or presence of symptoms [22]. Meta-analyses have revealed that 30-day or in-hospital all-cause mortality is lower after elective endovascular aneurysm repair (EVAR) than after open surgical repair (OSR) [24], but that late-phase (5 years post-intervention) survival is significantly worse after EVAR than after OSR [25]. The long-term reintervention rate is greater for EVAR than for OSR [26], with the primary requirement for re-intervention being endoleaks after EVAR and incisional hernias after OSR [25].

The European Society of Cardiology recommends a tailored pharmaceutical approach to achieve the primary objectives of prevention of major cardiovascular events and reduction of AAA growth [2]. A number of studies have assessed the efficacy of anti-hypertensive agents (β-adrenoceptor antagonists, angiotensin converting enzyme (ACE) inhibitors, diuretics, Ca^{2+} channel blockers), antibiotics (macrolides, tetracyclines), lipid lowering drugs (statins) and anti-platelet agents (non-steroidal anti-inflammatory drugs) for reducing AAA expansion rate. However, following evaluation of randomized clinical trials, no statistically significant reduction in AAA growth was observed for patients who were treated with the anti-hypertensive agents [27, 28]. An association study that adjusted for variables that affect AAA growth showed no effect of statin therapy on small AAA expansion [29]. Controversy exists amongst studies examining the potential benefits of aspirin, an antiplatelet and anti-inflammatory agent, on AAA progression. Whilst one study identified a reduced aortic expansion rate in patients with medium-sized AAA (40-49 mm) following use of low-dose aspirin, small patient number was a limiting factor (n=14/17 aspirin/no aspirin) [30]. Other studies have concluded that aspirin does not reduce rate of progression [29, 31] or incidence of rupture [32]. Data extracted from the Danish National Registry regarding 8020 AAA patients examined the association between preadmission use of low-dose aspirin and risk of aortic rupture. After adjusting for potential confounding variables, the findings showed that low-dose aspirin provided no reduction in risk of aortic rupture. Furthermore, low-dose aspirin was associated with a higher 30-day case fatality rate [32]. Taken together, drug therapies that aim to reduce the rate of AAA progression have been disappointing, leading Kokje *et al*. [10] to conclude: “At this point, no

pharmaceutical therapy can be recommended for the stabilization of AAA". New treatment strategies are required. There has been recent interest in the potential of LC n-3 PUFAs to interfere with inflammatory and oxidative stress processes that are associated with AAA.

4. LC n-3 PUFAs: mechanisms of action

The richest natural source of the biologically active LC n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is seafood, especially oily fish [33]. A high dietary intake of LC n-3 PUFAs increases cellular phospholipid EPA and DHA levels to result in partial substitution of arachidonic acid, a net decrease in eicosanoid production and favourable impacts on inflammatory responses [34]. Quantitative metabolic data obtained from an essential fatty acid-deficient mouse fibrosarcoma cell line showed inhibition of Δ5-desaturation of dihomo-γ-linolenic acid to arachidonic acid by LC n-3 PUFAs [35]. In a trial involving consumption of LC n-3 PUFAs in amounts equivalent to four oily fish meals per week (mean intake of LC n-3 PUFAs, 1.87 g/day), the median ratio of arachidonic acid to EPA+DHA in blood mononuclear cells declined from 5.93 at baseline to 2.53 at 12 months [36]. This change in fatty acid ratio may affect eicosanoid production and result in altered inflammatory, platelet-aggregating and vasoconstrictive activities [34, 37, 38].

The percentage contribution of DHA and EPA to the total fatty acids present in erythrocyte membrane phospholipids (termed the omega-3 index), is a long-term marker for dietary LC n-3 PUFA consumption. The omega-3 index is indicative of LC n-3 PUFA levels in a range of tissues from human and animal sources, as well as whole blood and plasma phospholipids [39-43]. A lower level of concordance exists between the omega-3 index and some tissue sources and plasma free fatty acid, triacylglycerol and cholesteryl ester LC n-3 PUFAs [43, 44]. A high (≥8%), and low (<4%) omega-3 index is associated with a low and high risk of death from coronary heart disease, respectively [39]; with the former being cardioprotective. In addition to membrane incorporation and the downstream impacts on pro-inflammatory eicosanoid production, several alternate mechanisms have been identified as contributing to the positive impact of LC n-3 PUFAs on cardiovascular biology and

inflammatory mechanisms. These include activation of the G-protein-coupled receptors, FFA4 (previously GPR120), suppression of pro-inflammatory nuclear factor kappa B (NF-κB) via modulation of Toll-like receptor 4 (TLR4) signalling and activation of peroxisome proliferator-activated receptor gamma (PPARγ), and synthesis of anti-inflammatory and pro-resolution enzymatic lipid metabolites and peroxidation products of LC n-3 PUFAs (Figure 1).

4.1 Mechanisms of action: NF-κB

NF-κB is a transcription factor that modulates expression of pro-inflammatory and pro-atherogenic genes [34]. In the inactive form, NF-κB interacts non-covalently with inhibitory IκB proteins to ensure their sequestration in the cytoplasm [45]. Following binding of inflammatory signals to their receptors (e.g. lipopolysaccharide (LPS) binding to TLR4), a signalling cascade is initiated that culminates in the phosphorylation, polyubiquitination and proteosomal degradation of IκB and the release and nuclear translocation of NF-κB [46]. Within the nucleus, NF-κB binds to discrete promoter and enhancer DNA sequences to upregulate transcription of inflammatory cytokines, adhesion molecules, cyclooxygenase-2 and inducible nitric oxide synthase [47]. LC n-3 PUFAs, especially DHA, inhibit LPS-stimulated dimerization of TLR4 and its translocation to lipid rafts, microdomains within the cell membrane that link TLR4 with proinflammatory signalling molecules [48, 49]. The effect is likely mediated by an alteration in lipid membrane organisation, with DHA-containing phospholipids partitioning into lipid rafts and EPA into non-raft domains [50]. A study by Wong et al [48] investigated the mechanism by which DHA disrupts TLR4 signalling. LPS and the saturated fatty acid, lauric acid were found to stimulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and reactive oxygen species (ROS) production, while DHA inhibited this response. NADPH oxidase inhibitors, diphenyleneiodonium chloride and N-acetyl-L-cysteine inhibited recruitment of TLR4 into lipid rafts and TLR4 dimerization [48], raising the possibility that DHA might produce similar effects. There is some evidence to suggest that NADPH

oxidase is recruited to lipid rafts in neutrophils following cell stimulation, and that this is associated with efficient initiation of NADPH oxidase activity, albeit with no effect on maximal activity rate [51].

4.2 Mechanisms of action: PPARγ

LC n-3 PUFAs also attenuate inflammatory gene expression that is under the control of NF-κB by activating PPARγ [52]. Agonist occupation of PPARγ results in SUMOylation of the nuclear receptors, leading to prevention of co-repressor complex degradation [53]. Through this mechanism, LC n-3 PUFAs maintain target genes in a repressed state. In addition, LC n-3 PUFAs direct macrophage polarization towards an M2 phenotype [54], an effect that is also dependent on PPARγ activation, and culminates in increased macrophage efferocytosis and secretion of the anti-inflammatory cytokines IL-10 and TGF-β [54].

4.3 Mechanisms of action: FFA4

FFA4 is a target for LC n-3 PUFA-mediated suppression of pro-inflammatory NF-κB signalling [55]. Activation of macrophage FFA4 by LC n-3 PUFAs inhibits the phosphorylation of several kinases, resulting in prevention of IκB degradation and inhibition of TNF-α and IL-6 secretion [56]. LC n-3 PUFA-induced FFA4 activation promotes β-arrestin-2 recruitment and, following internalisation, the resulting FFA4/ β-arrestin-2 complex binds to transforming growth factor-β activated kinase 1 (TAK1) binding protein 1 (TAB1) which interferes with this molecule's binding, phosphorylation and activation of TAK1 [56]. In the absence of TAK1 activation, stimulation of the IKKβ/NF-κB and JNK/AP pathways fails to occur [56].

4.4 Mechanisms of action: specialised pro-resolving lipid mediators

The biological activities of lipid mediators produced by enzymatic oxygenation reactions involving EPA (E-series resolvins) and DHA (D-series resolvins, protectins, maresins), collectively referred to as "specialized pro-resolving lipid mediators" (SPMs) [57], represent an important anti-inflammatory mechanism by which LC n-3 PUFAs mediate their cardioprotective effects [58]. SPMs attenuate

neutrophil transendothelial migration, stimulate clearance of apoptotic polymorphonuclear leukocytes, regulate pro-inflammatory gene expression and modulate chemokine/cytokine production. The activities of these lipid mediators have been well defined and extensively reviewed [58-60].

4.5 Mechanisms of action: Nrf2

In a further mechanism, LC n-3 PUFAs may be oxidised to 4-hydroxy-2E-hexenal (4-HHE), a reactive aldehyde that binds Kelch-like ECH-associated protein 1 (Keap1) [61] to trigger the dissociation of Keap1 from nuclear factor E2-related factor 2 (Nrf2). This process allows nuclear translocation of Nrf2. Through an interaction with the antioxidant response element, Nrf2 upregulates expression of antioxidant enzymes such as heme oxygenase (HO-1) [61, 62].

5. Can LC n-3 PUFAs attenuate progression of AAA?

A high dietary intake of LC n-3 PUFAs is associated with a low risk for inflammatory disease mortality [63]. In support of the anti-inflammatory effects of LC n-3 PUFAs, a high dietary intake is associated with low circulating concentrations of C-reactive protein (CRP), IL-6 and TNFα [64, 65]. Since AAA is an inflammatory disease involving macrophage and neutrophil infiltration into the aortic wall, a high LC n-3 PUFA diet might also provide protective effects against disease progression. Although this supposition is yet to be tested in a clinical trial, it is of interest to note that the incidence of AAA is lower in a Japanese population (1.7%) [66], which has a high dietary intake of EPA+DHA (950 mg/day) [67] compared to a European cohort (incidence, 4.3-4.9%) [66], which has an approximately 4-fold lower dietary intake of EPA+DHA (250 mg/day) [67]. Whether other lifestyle differences contribute to this observation is not known. For example, smoking is a major risk factor for AAA [31, 68], and contributes to a low omega-3 index that is independent of dietary EPA+DHA [69]. Despite this, tobacco is unlikely to explain the differences in AAA incidence between the Japanese and European cohorts. The age-standardized prevalence of current tobacco smoking in males 15 years and older in Japan (33.7%) was similar to, or greater than that in Germany (32.4%), Spain (31.3%), France (29.8%),

Italy (28.3%), Belgium (26.5%), Hungary (24.8%), the United Kingdom (19.9%) and Switzerland (19.7%) [70].

Coronary artery disease and low serum high density lipoprotein concentration are associated with AAA [71]. Although fasting serum low density lipoprotein and triglyceride levels show no association with AAA [71], imaging mass spectrometry has revealed elevated triglyceride levels in adipocytes within aneurysmal sac adventitia compared to a non-aneurysmal region of the abdominal aorta [72]. While high doses of LC n-3 PUFAs have been shown to reduce serum triglyceride levels [73], it is not yet known whether this treatment regimen would also lead to reduced tissue adipocyte triglycerides or abrogate tissue inflammation in AAA.

Randomized, placebo-controlled clinical trials investigating the prophylactic effect of LC n-3 PUFA supplementation in patients with small AAA are warranted. The only trial to date that has investigated the effect of LC n-3 PUFAs in human AAA examined a fish oil-containing emulsion supplement administered for 4 days immediately following AAA repair surgery [74]. Patients received 0.15 g/kg fat per day as an emulsion containing medium and LC triglycerides in a 50:50 ratio, or medium and LC triglycerides with added fish oil in a 40:50:10 ratio. As expected, patients with the fish oil supplementation had higher plasma EPA and DHA levels than non-treated control subjects. CRP levels increased at days 3 and 4 after surgery in the non-fish oil-treated group, and fish oil failed to protect against this response. Other end-points such as altered glucose metabolism, plasma malondialdehyde concentration and trolox equivalent antioxidative capacity were also unaffected by fish oil supplementation. Other biomarkers of inflammation and oxidative stress, such as 8-isoprostane and plasma cytokine concentrations, were not investigated. The authors raised the possibility that a small difference in fatty acid composition between the treatment and control groups might have negated positive outcomes in the fish oil group [74]. Notably, the study involved a short LC n-3 PUFA treatment duration. In a study in which participants ingested 0.51 g/day DHA, erythrocyte phosphatidylcholine DHA content at day 4 was 14-34% of that at day 29 [75], indicating

the importance of duration of LC n-3 PUFA supplementation. If incorporation of fatty acids into membrane phospholipids is a critical determinant of the LC n-3 PUFA effect, then sub-optimal incorporation may have resulted from the short-duration protocol in the trial in patients undergoing AAA repair [74]. The type and dose of LC n-3 PUFAs should also be considered, as efficiency of fatty acid incorporation into membrane phospholipids is different for EPA and DHA, and is dose-dependent [76].

6. Specialized pro-resolving lipid mediators and AAA

Prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2), together with the leukotrienes increase microvascular permeability, and stimulate platelet activation and neutrophil chemotaxis, all of which contribute to the initiation of inflammation [77, 78]. An acute inflammatory response that is distinct from adventitial AAA inflammation is initiated during open repair surgery, within 5 minutes of unclamping of the infrarenal aorta [57]. This response is characterised by increased leukotriene B_4 (LTB_4) and PGE_2 levels within the inflammatory exudate. In time, the inflammation resolves through an active process involving the formation of SPMs [57]. SPMs, with elevated levels at 72 hours post-surgery, initiate resolution that is characterized by attenuated neutrophil infiltration, reduced production of neutrophil-derived reactive oxygen species, and efferocytosis [57]. The switch from prostaglandin, thromboxane and leukotriene production to SPMs demarcates the transition from inflammation to resolution [78] and PGE_2 has been identified as a molecule that coordinates this response [79]. Notwithstanding this role of PGE_2, higher levels of cyclooxygenase-2 and PGE_2 are expressed in AAA compared to non-AAA aortas [80, 81]. Consistent with a pro-inflammatory role of PGE_2 in AAA, the EP4 prostanoid antagonist ONO-AE3-208 inhibited MMP-2 activation and IL-6 production in human AAA explants [82]. Anti-inflammatory drugs are known to inhibit cyclooxygenase, abolishing secretion of PGE_2 from AAA aortic explant cultures [81]. These drugs have been associated with slowed aneurysm growth rate in AAA patients [81]. This beneficial effect is unlikely to be associated with low-dose aspirin as patients in both case (20%) and control (33%)

groups reported use of this class of drug. Concerns over the use of cyclooxygenase inhibitors in preventing resolution are somewhat allayed by evidence that the inhibitors stimulate production of 15-epi-LXA_4 (aspirin-triggered lipoxin) [83], which has pro-resolving activity and the ability to reduce pro-inflammatory cytokine production [81].

In the event that resolution fails to initiate, a state of chronic inflammation ensues [84]. Potential benefits of pro-resolving metabolites of the LC n-3 PUFA DHA (resolvins D1 and D2) in AAA have been proposed. In an angiotensin II-infused $ApoE^{-/-}$ mouse model of pre-AAA, plasma resolvin D1 concentration was significantly greater in mice receiving dietary supplementation with cereal containing high (total LC n-3 PUFA, 0.7%) compared to a low LC n-3 PUFA content (total LC n-3 PUFA, 0.14%) [85]. Plasma resolvin D1 in C57BL/6 control mice did not change, suggesting that an inflammatory stimulus together with an elevated LC n-3 PUFA nutritional status is required to drive resolvin D1 production. Resolvin D1 may provide protective effects in the aorta by modulating fatty acid metabolism within infiltrating macrophages. In cultured bone marrow derived macrophages, resolvin D1 inhibited LTB_4 and enhanced LXA_4 production following exposure of cells to arachidonic acid [78]. In an elastase-perfusion mouse model of AAA, administration of resolvin D1 or D2 (100 ng/kg/treatment) lead to a significant reduction in AAA development and lower concentrations of pro-inflammatory cytokines compared to vehicle-infused controls [86]. Treatment with resolvin D2 also reduced inflammatory cytokine levels, decreased macrophage infiltration and lead to greater preservation of vascular elastin in angiotensin II-infused $ApoE^{-/-}$ mice [86].

7. Animal models of AAA

Scarcity of information on AAA etiology, attributed to limited availability of small AAA tissue, has necessitated the development of suitable animal models of the disease [87, 88]. Such models have included a broad range of mammalian and avian species with mouse models dominating biomedical research as a consequence of their ease of use, low cost, small size and well-characterised genomes [88, 89]. Common approaches to experimental murine aneurysm induction have included physical,

genetic and chemical manipulation of healthy aortic vasculature with the latter typically involving intraluminal elastase perfusion, periaortic $CaCl_2$ application or subcutaneous angiotensin II perfusion [90]. While interspecies variation in biology and physiology mean no single experimental model precisely mimics the human condition, animal-based studies provide significant insight into mechanisms of AAA, including aneurysmal degeneration to thereby contribute to the evaluation of novel therapeutic strategies to control and ameliorate AAA.

Notably, differences between human abdominal aortas and aortas of rodents commonly used as models of AAA have been reported previously. For example, the ratio of the number of medial elastic lamellar units to abdominal aortic wall thickness is lower in humans than in mice and rats [91]. Differences in AAA localization in humans (mainly infrarenal aorta) and $ApoE^{-/-}$ mice (mainly suprarenal aorta) have been attributed to species differences in hemodynamics and an associated effect of this on oscillatory shear stress [92]. The method for AAA induction in animal models also influences the type of human disease that is represented. For example, intraluminal administration of elastase to mice replicates many features of elastin degradation and chronic inflammation observed in human AAA, whereas extraluminal application replicates acute adventitial inflammation, with preservation of elastin and endothelial integrity [93]. A recent systematic review provides a detailed account of the advantages and limitations of commonly used animal models of AAA [88].

7.1 LC n-3 PUFAs: experiences with animal models of AAA

A number of studies have used animal models to investigate the effect of LC n-3 PUFAs or their metabolites on either early or late stages of AAA development (Table 1). These studies infused $ApoE^{-/-}$ mice with angiotensin II for 2-14 days to induce a pre-AAA inflammatory response [85, 94, 95] or for 4 weeks to induce AAA [96]. In one study, short-term $CaCl_2$ application to the periaortic region followed by 6 week maintenance of the animals facilitated AAA development [97], while another study perfused the abdominal aorta with elastase for 5 min, followed by maintenance of animals for 2 weeks [86]. AAA was also induced by 4 week ligation of the abdominal aorta in rats [98]. In this

work, supplementation of rodent diets with LC n-3 PUFAs, or injection of resolvins D1 or D2, protected animals against AAA development [86, 96-98]. While all of the animal studies investigated the preventive effects of LC n-3 PUFAs or their metabolites when administered prior to induction of AAA [85, 86, 94-98], two studies reported on the effects of LC n-3 PUFAs [96] or resolvin D2 [86] in animals with existing pre-AAA or small AAA.

$ApoE^{-/-}$ angiotensin II-infused mice that received a low LC n-3 PUFA dietary supplement had greater macrophage and neutrophil infiltration [85, 96] than mice that were supplemented with higher levels of LC n-3 PUFAs. Treatment of animals with LC n-3 PUFAs [96] or resolvin D2 [86], directed macrophage polarization within the aortic wall toward the M2 phenotype, which is associated with anti-inflammatory function [99]. Interestingly, an inhibitory effect on macrophage infiltration was not observed in $CaCl_2$ treated mice supplemented with EPA [97], possibly indicating a different etiology between mouse models. Work examining LC n-3 PUFA dietary supplementation consistently reports suppressed expression and activity of matrix metalloproteinases (MMPs). Higher levels of MMP-9 have been reported in AAA compared to non-AAA blood and aorta [100]. MMPs are expressed in luminal endothelial cells, macrophages and smooth muscle cells within human AAA aorta, and there is an inverse correlation between infiltrating inflammatory cells and aortic elastin content [101]. The ability of MMPs to degrade elastin has been widely reported [102-104] and LC n-3 PUFAs may protect the aorta by suppressing MMP elastolytic activity. In the short-term angiotensin II infusion animal studies, LC n-3 PUFAs supplementation decreased MMP-9 protein expression in inflammatory cells within the adventitia, and in aortic endothelial cells [94]. These effects were accompanied by reduced MMP-9 activity, but no change in MMP-2 expression. In studies with longer-term stimuli for AAA development, EPA supplementation decreased MMP immunoreactivity in smooth muscle cells (MMP-2) and macrophages (MMP-2 and MMP-9) [97]. Reduced levels of MMP-2 and MMP-9 gene expression [96, 97] and MMP activity were also observed in mice that were fed an EPA supplemented diet (MMP-2 activity [96], MMP-9 activity [96, 97]), or DHA (MMP-2 and MMP-9 activity [96]). Although the high LC n-3 PUFA diet lead to higher TIMP-1

immunoreactivity in inflammatory cells during short-term infusion with angiotensin II [94], no effect of EPA was observed at one and three weeks post-$CaCl_2$ application, suggesting either a time, or stimulus-dependent regulation of TIMP-1. Consistent with a vasoprotective effect of LC n-3 PUFAs via abrogation of MMP activity, dietary supplementation of mice with LC n-3 PUFAs produced a trend for reduced elastin fragmentation in pre-AAA mice [94], which reached significance in the $CaCl_2$-induced AAA mouse model [97]. Partial disappearance of elastic lamina was detected in the hypoperfusion rat model of AAA, however this was unaffected by fish oil intake [98]. Despite this finding, a significant reduction in MMP-2 and MMP-9-positive areas was reported in fish oil-fed rats, and it was speculated that other proteases might be important in elastin degradation in this model [98].

Several studies showed reduced infiltration of inflammatory cells into the aortic wall after supplementing the diet with LC n-3 PUFAs [85, 96] or after intraperitoneal injection of resolvin D1 or resolvin D2 [86]. The studies reported reduced production of pro-inflammatory cytokines [86, 96] and biomarkers of oxidative stress [85, 98] with LC n-3 PUFA or D-series resolvin treatment. In the $ApoE^{-/-}$ mouse model of AAA, the amount of superoxide produced per inflammatory cell was elevated in animals receiving the low LC n-3 PUFA diet compared to mice receiving the high LC n-3 PUFA diet [85]. The NADPH oxidase, NOX2 is a likely source of the superoxide, with a corresponding higher NOX2-like immunoreactivity in mice receiving the low- compared to the high LC n-3 PUFA diet [85].

8. Anti-hypertensive effects of LC n-3 PUFAs

Antihypertensive drugs do not affect AAA growth rate [23, 105, 106]. Although angiotensin II infusion in $ApoE^{-/-}$ mice initiated AAA development, this was not replicated in animals that were infused with noradrenaline, despite both agents causing similar increases in blood pressure [107]. Normalization of blood pressure with hydralazine in the angiotensin II-infused mice, or administration of a sub-pressor dose of angiotensin II failed to prevent aneurysm formation,

consistent with a mechanism for AAA growth that is independent of blood pressure [107]. However, hypertension is a positive risk factor for AAA rupture [105], and ACE inhibitors have been shown to have a protective effect with an associated 17% reduction in odds for rupture [106]. Although the prevalence of AAA is lower in women than in men, women with aortic aneurysm have a significantly higher risk for AAA rupture [105]. In a prospective population-based study of patients with an acute vascular event affecting the aorta, hypertension was identified as a major risk factor for incidence of AAA in women, leading the authors to recommend active management of hypertension in women [108]. It remains to be seen whether LC n-3 PUFAs, which contribute to blood pressure reduction ([109] for review), might provide some protection in women with AAA through this mechanism.

9. Conclusion

AAA is a progressive, inflammatory disease that carries an increased risk for aortic rupture. Drug therapies trialled to date lack efficacy, with patients managed through careful surveillance of aneurysm development and, for patients with larger aneurysm, repair surgery. It is hypothesised that the anti-inflammatory and pro-resolving effects of LC n-3 PUFAs and their metabolites will reduce the burden of inflammatory infiltrate into the aortic wall and thereby slow the progression of disease. While this supposition has yet to be tested in human clinical trials, animal studies have provided some potential insight into the efficacy of interventions involving pharma-nutritional administration of these bioactive compounds. Reported benefits include reduced rate of aneurysm development, and reduced infiltration of inflammatory cells, cytokine production, MMP expression and activity, and elastin degradation. Congruence of findings across multiple, well-established animal models, including intraluminal elastase perfusion, periaortic $CaCl_2$ application, subcutaneous angiotensin II perfusion, and aortic hypoperfusion, is encouraging. Although studies with LC n-3 PUFAs have been primarily limited to rodent models of AAA, the findings outlined here should provide the necessary impetus for expansion towards human clinical trials.

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Figure 1 Mechanisms contributing to the anti-inflammatory and antioxidant effects of LC n-3 PUFAs. 1. substitution of LC n-6 PUFAs with LC n-3 PUFAs; 2. activation of FFA4 receptors; 3. disruption to membrane organisation culminating in interference in TLR4 dimerization; 4. activation of PPARγ to sustain repression of NF-κB; and 5. synthesis of anti-inflammatory and pro-resolution enzymatic lipid metabolites and peroxidation products of LC n-3 PUFAs. AA, arachidonic acid; β-arr2, β-arrestin2; COX, cyclooxygenase; DHA, Docosahexaenoic acid; Δ5-D, Delta-5 desaturase; DH-γLA, Dihomo-γ-linolenic acid; FFA4, Free fatty acid 4 receptor; HO-1, Heme oxygenase-1; 4-HHE, 4-Hydroxy hexenal; IKβ, Inhibitor of kappa B; IKKβ, Inhibitor of kappa B kinase; iNOS, Inducible nitric oxide synthase; IRAK, Interleukin-1-receptor-associated kinase; Keap1, Kelch-like ECH-associated protein 1; LC n-3 PUFA, Long chain omega-3 polyunsaturated fatty acids; LOX, lipoxygenase; LPS, lipopolysaccharide; LTB_4, Leukotriene B_4; MD-2, Myeloid differentiation factor 2; MyD88, Myeloid differentiation primary response 88; NF-κB, Nuclear factor kappa B; NOX2, Nicotinamide adenine dinucleotide phosphate-oxidase 2; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; $p22^{phox}$, Human neutrophil cytochrome b light chain; $p47^{phox}$, Neutrophil cytosol factor 1; PGE, Prostaglandin E; PLA2, Phospholipase A2; PPARγ, Peroxisome-proliferator-activated receptor gamma; SPMs, Specialized pro-resolving lipid mediators; Su, Small ubiquitin-like modifiers; TAB1, Transforming growth factor-beta activated kinase 1 binding protein 1; TAK1, Transforming growth factor beta-activated kinase 1; TLR4, Toll-like receptor 4; TRAF6, Tumour necrosis factor-receptor-associated factor 6; Ubiq, Ubiquitination.

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