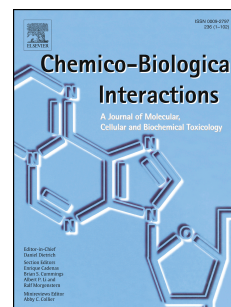


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Insights into the Cardioprotective Properties of n-3 PUFAs Against Ischemic Heart Disease Via Modulation of the Innate Immune System

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Abstract

Ischemic heart diseases (IHD) is a major cause of cardiovascular death and disability worldwide. IHD is characterized by an imbalance between cardiac oxygen supply and demand predominantly due to obstruction of coronary arteries. Activation of the innate immune system and the consequent inflammatory response is an important contributor in the pathogenesis of IHD. An excessive and uncontrolled inflammatory response contributes to the adverse cardiac remodeling and fibrosis, making inflammation an important therapeutic target for improving outcomes in the cascade of IHD. While there are many discrepancies in the literature, evidence from both bench and clinical research demonstrate the beneficial effects of increased n-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA), toward IHD. N-3 PUFA, and their metabolites, have been demonstrated to modulate different aspects of the immune system, including the expression of adhesion molecules, cytokines, leucocyte chemotaxis and inflammasome formation. In this article, we provide a brief overview of the role of the innate immune system in IHD and focus on the immunomodulatory effects of n-3 PUFAs and their biologically active metabolites.

Key words: N-3 polyunsaturated fatty acids; bioactive lipid mediators; innate immune system; ischemic heart disease

List of Abbreviations: AA, Arachidonic acid; AAR, Area at risk; ACS, Acute coronary syndrome; AHA, American Heart Association; ALA, α -Linolenic acid; ASC-1, Apoptosis-associated speck-like protein containing a CARD-1; CAD, Coronary artery disease; CB2R, Cannabinoid receptor 2; CCL, Chemokine ligand; CCR, Chemokine receptor; CHD, Coronary heart disease; COX, Cyclooxygenase; CRP, C-reactive protein; CYP, Cytochrome P450; CysLTs, Cysteinyl leukotrienes; CVD, Cardiovascular disease; DAMP, Damage-associated molecular patterns; DGLA, Dihomo- γ -linolenic acid; DiHDDPA, Dihydroxydocosapentanoic acid; DHA, Docosahexaenoic acid; DHEQ, Dihydroxyeicosatetraenoic acid; EDP,

Epoxydocosapentaenoic acid; EET, Epoxyeicosatrienoic acid; EEQ, Epoxyeicosatetraenoic acid; EP, E prostanoid receptor; EPA, Eicosapentaenoic acid; GPR, G-protein coupled receptor; HDoHE, Hydroxy docasahexaenoic acid; HEPE, Hydroxyeicosapentaenoic acid; HETE, Eicosatetraenoic acid; HF, Heart failure; HFHC, High-fat/high-carbohydrate; HMEC, Human microvessel endothelial cells; HMGB-1, High mobility group box-1; HpDHA, Hydroperoxydocosahexaenoic acid; HpETE, Hydroperoxyeicosatetraenoic acid; HSP, Heat shock protein; HUVEC, Human umbilical vein endothelial cells; ICAM, Intracellular adhesion molecule; IFN, Interferon; IGF-1, Insulin-like growth factor 1; IHD, Ischemic heart disease; IL, Interleukin; IR, Ischemia-Reperfusion; LA, Linoleic acid; LAD, Left anterior descending coronary artery; LDL, Low-density lipoprotein; LID, Lingui Zhugan Decoction; LOX, Lipoxygenase; LT, Leukotriene; LV, Left ventricle; MaR, Maresin; MCP-1, Monocyte chemoattractant protein-1; MI, Myocardial infarction; miR, MicroRNA; MMP, Matrix metalloproteases; MPTP, Mitochondrial permeability transition pore; mtDNA, Mitochondrial DNA; NF- κ B, Nuclear factor kappa-light-chain enhancer activated B-cells; NLRP3, NACHT, LRR and PYD domains-containing protein 3; PD, Protectin; PG, Prostaglandin; PLA2, Phospholipase A2; PMN, Polymorphonuclear neutrophils; PPAR, Peroxisome proliferator-activated receptor; PPCI, Primary percutaneous coronary intervention; PUFA, Poly unsaturated fatty acid; ROS, Reactive oxygen species; Rv, Resolvin; TAC, Transverse aortic constriction; TAK, TGF-activated kinase; TGF- β , Transforming growth factor- β ; TIMP, Tissue inhibitor of metalloproteinase; TLR, Toll like receptor; TNF- α , Tumor necrosis factor- α ; Tx, Thromboxane; VCAM, Vascular adhesion molecule; VSMC, Vascular smooth muscle cells.

1. What is ischemic heart disease

Ischemic heart disease (IHD) is the most common type of heart disease and is a leading cause of cardiovascular morbidity and mortality worldwide [1-5]. IHD is the term applied to a group of closely related syndromes resulting from myocardial ischemia. Ischemia usually arises due to the imbalance or mismatch between myocardial demand and supply by the oxygenated blood. Beside insufficiency of oxygen, ischemia also comprises reduced nutrient availability and impaired removal of metabolic waste products. Several factors can contribute to increasing cardiac oxygen demand such as increased heart rate, contractility and elevated blood pressure. While compromised perfusion of the heart is usually attributed to decreased coronary blood flow, coronary vasospasm or hypotension, as well as reduced hematocrit and blood oxygen saturation. In more than 90% of cases, the cause of myocardial ischemia is the reduction in blood flow to the heart due to narrowing or even complete obstruction of the coronary arteries by excessive lipid accumulation and consequently the formation of atherosclerotic plaque on the vessel wall. Therefore, IHD is often termed coronary artery disease (CAD) or coronary heart diseases (CHD) interchangeably [6-8].

Angina pectoris is the most common manifestation of IHD where ischemia is less severe and does not cause death of the cardiac muscle, however, it starts to delineate the area at risk (AAR) of potential death. If the duration and severity of ischemia is prolonged (more than 20 minutes) a “wave front” of irreversible cardiomyocyte death throughout the myocardium arises and spreads from the subendocardium to the subepicardium, a case known as myocardial infarction (MI) [9]. Acute MI often results from sudden interruption of blood flow downstream of the blocked vessel due to the rupture or erosion of an unstable atherosclerotic plaque with a consequent thrombus formation in coronary arteries. This acute cardiac insult is often referred to as acute coronary syndrome (ACS) [10-12].

After MI, early and successful myocardial reperfusion or restoration of blood flow to the ischemic myocardium, using either thrombolytic therapy or primary percutaneous coronary intervention (PPCI), is the standard therapeutic intervention to rescue viable myocardial tissue, reduce infarct size, and decrease acute mortality rates. However, reperfusion itself can paradoxically accelerate the death of injured cardiomyocytes, aggravating the damage and increasing the incidence of chronic heart failure (HF). This phenomenon termed as ischemia-reperfusion (IR) injury may account for up to 50% of the final infarct size [13-17] and explain why despite optimal myocardial reperfusion, the death rate after an acute MI still approximates 10% [18]. The mechanisms underlying reperfusion injury are complex, involving multiple factors such as mitochondrial dysfunction [19], opening of the mitochondrial permeability transition pore (MPTP) [20], generation of reactive oxygen species (ROS) fueled by rapid reintroduction of molecular oxygen [21], endoplasmic reticulum stress [22], calcium overload [20, 23-25], endothelial dysfunction and inflammation [26, 27]. The increased injury contributes to the further development of MI, which results in marked cardiac remodeling, ultimately leading to heart failure [28-31]. A better understanding about the pathophysiology of IHD remains important, notably toward the development of more effective therapeutic strategies.

2. Role of innate immune system in the pathogenesis of ischemic heart disease

The innate immune system is considered the first line of the body's defense against injury and insult, which is characterized by an ability to detect and respond quickly to both invading pathogens and sterile cell stressors. The innate response is triggered by the activation of a group of receptors called pattern recognition receptors (PRRs) found on the surface of neutrophils, monocytes/macrophages, endothelial cells and cells in the injured tissue. PRRs are activated following binding of specific microbial motifs or endogenously generated danger signals. Danger signals most commonly stem from cellular debris or intracellular products released early from the injured or damaged cells [32-36]. PRR activation quickly initiates an

immune response within the insulted tissue and the whole body by triggering the migration of additional innate immune cells to the affected site, inducing the production of mediators needed for inflammation and repair as well as alerting, instructing and activating the more specific adaptive immune response mediated by T and B lymphocytes [37-41]

Accumulating literature supports the association between the sterile activation of the innate immune system and IHD [42-45]. The incidence of infarction in the myocardium activates the inflammatory reaction which involves two mechanistically distinct phases, the inflammatory phase and the reparatory phase. Death of cardiomyocytes under acute ischemic conditions triggers the initial pro-inflammatory response to first remove necrotic cellular debris from the infarct zone and start the reparative phase. Reperfusion of the ischemic myocardium contributes to tissue loss by accelerating the death of the injured cardiomyocytes exacerbating the pro-inflammatory response and increasing the size of the infarct zone [34, 46-48]. The early pro-inflammatory response is followed by a reparative phase involving resolution of inflammation, myofibroblast proliferation, wound healing and scar formation [49]. Whether these repair mechanisms are beneficial or detrimental to cardiac function is partially dependent upon the amount of tissue damage [50]. Accordingly, persistent or extended inflammatory phase responses can exaggerate myocardial damage leading to an increase in the infarct size and excessive cardiac remodeling [51]. Treatments targeting the innate immune response may provide a promising therapeutic strategy for limiting infarct size, ameliorating adverse remodeling and improving cardiac function. In this section we will discuss the role the innate immune system has in the pathogenesis and the progression of IHD.

2.1. Inflammatory response in coronary atherosclerosis

CAD is a chronic inflammatory fibroproliferative disease characterized by abnormal lipid metabolism and buildup within the vascular wall of the coronary arteries associated with a potent inflammatory pathophysiological reaction [52-54]. Atherosclerosis is a multistep process

initiated by subendothelial oxidation of low-density lipoproteins (LDL), followed by infiltration of monocytes and their conversion to macrophages and then to lipid-laden foam cells, proliferation of vascular smooth muscle cells (VSMCs) and finally secretion of fibrous elements leading to the formation of occlusive plaques [53]. The high content of inflammatory cells such as neutrophils and monocytes present in the atherosclerotic plaque are a direct source of many pro-inflammatory mediators such as chemokines, cytokines and leukotrienes (LTs), which worsen the inflammatory status [52, 54, 55]. Importantly, the inflammatory macrophages residing within the blood vessel wall can also release matrix metalloproteases (MMPs) that digest the fibrous cap of the atherosclerotic plaque. This leads to plaque rupture and a further cascade of inflammatory events with deleterious cardiovascular effects such as thrombi formation and coronary artery blockage impeding the blood flow to the heart leading to acute MI [56, 57].

2.2. Inflammatory response accompanying myocardial infarction

2.2.1 Activation of PRRs by damage-associated molecular patterns (DAMPs) (early signaling)

Cardiomyocyte cell death resulting from acute ischemic conditions or reperfusion injury causes the release of cellular debris and contents, referred to as damage-associated molecular patterns (DAMPs, alarmins). Some of these endogenous products can activate the innate immune response in adjacent myocardial cells, myofibroblasts, endothelial cells and migrating immune cells. DAMPs include protein signals such as high mobility group box-1 (HMGB1), S100 proteins or heat-shock proteins (HSPs) and non-protein signals ATP, mitochondrial DNA (mtDNA) and RNA [58-60]. PRRs sense extracellular threats through DAMPs to prime cells for a response to potential injurious conditions. Critical PRRs involved IHD are toll-like receptors 2 and 4 (TLR2 and TLR4), which are present on various cell types including cardiac, endothelial and circulating immune cells [34, 35, 50, 61-64]. Binding of DAMPs to TLR2 or TLR4 starts the

pro-inflammatory phase by activating NF- κ B signaling events. Once activated, NF- κ B translocates to the nucleus driving the expression and release of pro-inflammatory proteins and cytokines including tumor necrosis factor- α (TNF- α), pro-interleukin (IL)-1 β , pro-IL-18, IL-6, IL-8, CXC chemokines (neutrophil chemoattractants), CC chemokines (monocytes and T-lymphocytes chemoattractants) and cell adhesion molecules (e.g., vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM) and selectins) [65-69]. These mediators promote endothelial activation and permeability, leading to further sequential recruitment of neutrophil and monocytes to the injured myocardium [70, 71] (Fig. 1).

Acute MI can lead to an exaggerated activation of the inflammasome pathway spreading an inflammatory surge to the rest of the myocardium impacting cardiac function. Binding of DAMPs to TLRs or NOD-like receptors (NLR) on cardiac fibroblasts, infiltrating leucocytes and cardiomyocytes will activate the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome, a main pro-inflammatory mediator in the setting of MI [72-74]. NLRP3 inflammasomes are large multiple protein complex found in the cytosol that consists of a sensor protein (NLR), an adaptor protein (apoptosis-associated speck-like protein containing a CARD-1 (ASC-1)) and a zymogen (procaspase-1) [75]. Once aggregated, NLRs and ASC-1 mediate the cleavage and activation of caspase-1. Active caspase-1 then induces the conversion of pro-IL-1 β and pro-IL-18 to mature IL-1 β and IL-18 respectively, which induces pyroptosis or caspase-1 mediated cell death [74, 76-83]. The release of IL-1 β from cardiac fibroblasts, in response to MI requires two signals: (1) the transcription of pro-IL-1 β by the TLR-NF- κ B pathway, and (2) the activation of pro-IL-1 β to its mature form by the NLRP3 inflammasome. IL-1 β triggers the release of other cytokines and chemokines, which recruit and activate inflammatory cells such as neutrophils and monocytes. In general, IL-1 β is considered a major cytokine mediating the pro-inflammatory response post-MI [84, 85] (Fig. 1).

2.2.2 Recruitment of different leukocyte populations

The infiltration of the injured myocardium by inflammatory leukocytes is a well-organized process with the chronological recruitment of neutrophils, monocytes and macrophages that has been well documented [86, 87]. The pro-inflammatory and reparative roles of these immune cells in the setting of IHD may have different effects attributable to the functional variation and the amount time the immune cells reside in the injured tissue. As such, the role of leukocytes in the pathogenesis of IHD and post-MI healing may be viewed as a double-edged sword. For example, monocyte/macrophage recruitment is essential for post-MI infarct healing; however, uncontrolled and extensive infiltration may worsen the injury or impair reparative capabilities [88, 89]. Thus, the challenge is how to ameliorate the detrimental effects of these cells, while maintaining their beneficial reparative roles.

2.2.3 Neutrophils (1-3 days post-MI)

Neutrophils are the first immune cells to infiltrate the injured myocardium post-MI. They are recruited from the bone marrow within the first hours of injury and reach a peak after one day before slowly declining [90-92]. Cytokines and chemokines, such as cytokine-induced neutrophil chemoattractant 1 (CINC-1/CXCL1), LTB₄ and IL-8 (CXCL8), produced early in the inflammation cascade promote endothelial activation, permeability and neutrophil recruitment to the infarcted area. The polymorphonuclear neutrophils (PMNs) enter the insulted myocardium by adhering to and rolling on endothelial cells by binding to the cell adhesive molecules P-selectin, E-selectin, VCAM, and ICAMs expressed on activated endothelial cells [70]. Once in the injured myocardium, neutrophils start to remove necrotic cells and tissue debris; however, activated neutrophils can release high levels of ROS, DAMPs, proteolytic enzymes and secrete chemotactic factors further aggravating tissue damage. Excessive neutrophil infiltration and/or their delayed removal may exacerbate myocardial injury by prolonging the pro-inflammatory response [93-96]. Evidence from clinical studies suggests high levels of neutrophils and their products are correlated to the severity of IHD and infarct size post-MI [97].

2.2.4 Monocytes (3-5 days post-MI)

Nahrendorf et al. demonstrated a sharp increase in the number of inflammatory monocytes found in blood within the first few hours after coronary blockade reaching a peak 3-5 days post-MI in the injured heart [98]. Monocytes are recruited to the injured myocardium by the increased cardiac and endothelial expression of the chemoattractant chemokine monocyte chemoattractant protein-1 (MCP-1, also called chemokine ligand 2 (CCL2)) and neutrophil-derived granular protein cathelicidin [99-101]. Binding of monocytes and macrophages to CCL2 through their cell surface receptor CC chemokine receptor 2 (CCR2) can induce the expression of other cytokines, MMPs and transforming growth factor- β (TGF- β) causing further cardiac cell death and ventricular dysfunction contributing to injuries [102-104]. IL-1 β is an important stimuli of monocyte recruitment by triggering its production in the spleen and bone marrow following ischemic injury [105-107]. Monocytes and their lineage-descendant macrophages contribute to the resolution of the inflammatory response and ventricular remodeling, yet excessive monocytosis in the post-MI inflammatory period is deleterious for long-term cardiac function [87, 108, 109]. A large influx of monocytes can contribute to the initial cardiac injury and participate in the release of several inflammatory mediators, proteolytic enzymes and increased ROS production, exacerbating the pro-inflammatory phase.

2.2.5 Macrophages (5-7 days post-MI)

During the first days post-MI, the majority of cardiac macrophages are derived and replenished from inflammatory monocytes differentiating into classical M1 inflammatory macrophages that clear the cellular and matrix debris through efferocytosis [110]. Subsequently, alternatively activated or reparatory M2 macrophages are formed to promote resolution of inflammation and contribute to wound healing [98, 111, 112]. Controlled recruitment of macrophages to the injured myocardium is essential for wound healing and tissue repair as defective macrophage clearance of necrotic or apoptotic cells can lead to impaired collagen

deposition and scar formation, causing adverse left ventricular remodeling [113-116]. Excessive or prolonged residence of inflammatory M1 macrophages in the infarct myocardium can extend the inflammatory phase and consequently expand the infarcted area, delaying the reparative phase and formation of scar tissue mediated by M2 macrophages and thus aggravates the adverse cardiac remodeling [88, 89, 117].

2.3. *Persistent post-MI adverse inflammatory response and LV remodeling*

Cardiac remodeling and progression to HF following MI is dependent on the extent and persistence of the inflammatory response. Following MI, an excessive pro-inflammatory response may induce geometric and functional changes in the LV, which includes hypertrophy of the non-infarcted segments and dilatation of the infarcted segments worsening cardiac function [118]. Modulating the persistent or chronic inflammatory response can limit adverse LV remodeling.

During the healing process, the infiltrated macrophages and fibroblasts are responsible for the sustained upregulation of TGF- β , a key mediator in mediating LV remodeling, with its downstream effectors in the myocardium promoting fibrosis and remodeling of the injured cardiac tissue [119, 120]. Active TGF- β binds to its receptor (T β R) at the cell surface and propagates downstream intracellular signals through Smad proteins [121, 122]. Expression of the stimulatory Smad 2, 3, and 4 proteins were shown to be significantly upregulated under MI conditions, while expression of the inhibitory Smad7 is decreased in myocardial scars [123, 124]. The activation of the Smad3 signaling pathway mediates extracellular matrix protein synthesis and deposition in the non-infarcted myocardium as well as promotes matrix preservation through increased expression of tissue inhibitors of metalloproteinases (TIMP). TGF- β also activates TGF- β -activated kinase 1 (TAK1), a potent mediator of cardiomyocyte hypertrophy [119, 125]. Overall, TGF- β -mediated effects contribute to both excessive matrix

deposition and pathological hypertrophy post-MI, leading eventually to dilative cardiac remodeling and severe cardiac dysfunction.

3. *Modulation of the innate immune response is cardioprotective*

Increasing evidence in the literature demonstrates modulating the innate immune system can limit adverse consequences resulting from ischemic injury. Genetic or pharmacological inhibition of TLR2 or TLR4 was demonstrated to blunt the excessive inflammatory response post-MI and attenuate infarct expansion. For example, TLR4-deficient mice sustained smaller infarctions and exhibited less inflammation after myocardial IR injury [126]. Additionally, post-MI hearts in TLR2^{-/-} mice performed better than WT counterparts and were protected against endothelial dysfunction [127]. Interestingly, impaired TLR2 or TLR4 signaling prevented adverse cardiac remodeling and resulted in preserved cardiac function and geometry following MI [128, 129]. Very recently, Yuan et al., showed vaspin, a visceral adipose tissue-derived serine protease inhibitor adipokine, limits the infarct size post IR injury via inhibiting TLR4/NF- κ B signaling pathway both in vivo and in vitro [130]. In addition, it has been demonstrated that atazanavir, an antiretroviral medication, protects against MI-induced cardiac fibrosis through blocking HMGB1/TLR9 inflammatory signaling pathway in rat hearts [131]. Tanshinone IIA, the main effective component of the Chinese medicine Danshen, has also shown to attenuate MI progression and prevent LV remodeling through inhibition of TLR4/MyD88/NF- κ B signalling pathway in an acute MI rat model [132].

Targeting the NLRP3 inflammasome signaling pathway to inhibit different components (i.e. caspase 1, IL-1 β , ASC-1, or NLRP3 protein) has been demonstrated to reduce infarct size and preserve cardiac function in different models of MI [80-82, 133-136]. Different models support this notion, including evidence demonstrating the cardioprotective effects of cannabinoid receptor 2 (CB2R) agonists involves suppression of NLRP3 inflammasome activation [137]. While Wang et al., provided evidence the hormone Ghrelin protects the heart

against IR injury by inhibiting the TLR4/NLRP3 inflammasome pathway [138]. Other research suggests inhibiting CCL2/CCR2 signaling might blunt excessive recruitment of pro-inflammatory monocyte/macrophage, promote infarct healing, diminish interstitial fibrosis, prevent detrimental remodeling and consequently attenuate contractile dysfunction in the setting of MI [104, 139-141]. Very recently, Wang et al., demonstrated blocking monocyte migration to the infarcted myocardium post-MI with a CCR2 antagonist improved cardiac function and limited the infarct size [142].

Modulating macrophage polarization provides a strategy for reducing infarct size, preventing adverse LV remodeling and preserving cardiac function post-MI. Targeting either pro-inflammatory M1 macrophages or promoting M2 macrophage polarization can facilitate resolution of inflammatory responses and prevent adverse LV remodeling following MI [143-145]. Heinen et al., demonstrated short-term treatment with insulin-like growth factor 1 (IGF1) after acute MI increased the number of the anti-inflammatory M2 macrophages in heart tissue reducing infarct size and improving cardiac function [146]. Moreover, treatment with exogenous IL-19 attenuated acute ischemic injury and improved survival of mice following MI via inhibition of macrophage polarization toward the proinflammatory M1 phenotype while stimulating the polarization and formation of the pro-healing M2 macrophages [147]. Recent evidence suggests the type 2 diabetes mellitus medication, pioglitazone, can limit cardiac remodeling caused by IR injury or ligation of left anterior descending artery (LAD) by antagonizing monocyte/macrophage-mediated acute inflammation promoting cardiac healing. Pioglitazone reduced macrophage recruitment to the injured myocardium and promoted polarization of existing macrophages toward a M2 phenotype [148].

Several lines of evidence indicate inhibiting the activation of TGF- β and its downstream signaling pathways protects the heart against post-MI cardiac remodeling and fibrosis [149, 150]. For example, the cardioprotective effects of some traditional Chinese medicines, Linggui

Zhugan Decoction (LZD) and Qiliqiangxin, are attributed to inhibition of TGF- β 1/Smad-mediated signaling, which reduced myocardial inflammation limiting ventricular remodeling induced by MI [151]. Further evidence, demonstrated downregulating microRNA-330 (miR-330) inhibited the activation of the TGF- β 1/Smad3 signaling pathway suppressing LV remodeling in mice subjected to IR injury [152]. In contrast, microRNA-20b-5p promoted ventricular remodeling following myocardial IR injury in rats by inhibiting the expression of the inhibitory Smad7 by activating TGF- β 1/Smad signaling pathway [153]. Together, these studies highlight a role the innate immune system in the development and progression of MI.

4. Overview of n-3 and n-6 polyunsaturated fatty acids

The long-chain n-3 and n-6 polyunsaturated fatty acids (PUFA) are essential fatty acids obtained from dietary sources. They are characterized by the presence of their first double bond at the third (n-3 PUFA) or the sixth position (n-6 PUFA) starting from the omega carbon. The simplest n-3 PUFA is α -linolenic acid (ALA, 18:3 n-3) while linoleic acid (LA, 18:2 n-6) is considered the primary source of the essential n-6 PUFAs. Once inside the body, ALA and LA can be converted into other n-3 and n-6 PUFAs, respectively through a series of elongation and desaturation reactions (Fig. 2 and 3). For instance, ALA is metabolized into eicosapentaenoic acid (EPA, C20:5n-3) which can be further metabolized into docosahexaenoic acid (DHA, C22:6n-3). Using the same series of elongase and delta-4,-5,-6 desaturase enzymes, LA can be converted to dihomo- γ -linolenic acid (20:3n-6; DGLA) and metabolized further to yield arachidonic acid (AA, 20:4n-6). Mammals lack the necessary enzymes (delta-12 and delta-15 desaturase) required to synthesize and interconvert between LA and ALA de novo, as such these fatty acids are described as “essential” and must be obtained from the diet [154, 155]. The average daily intake of LA and ALA in Western countries is 10 and 1 g/day, respectively [156]. Importantly, both n-3 and n-6 PUFA compete for the same metabolic enzymes, however, metabolites generated from n-6 PUFAs are predominant as LA is more abundant in western

diets than ALA [157-159].

5. Cardiovascular benefits of n-3 polyunsaturated fatty acids

Early evidence suggesting cardiovascular benefits were associated with n-3 PUFAs originated from epidemiological studies in Greenland Inuit. These studies suggested a higher proportion of EPA compared to AA in their blood was associated with a lower incidence of MI compared to Danish study participants. It was hypothesized that these differences were due to the higher dietary intake of food sources rich in n3-PUFAs in the Greenland Inuit population [160, 161]. Since then, numerous studies have suggested a role of n-3 PUFA for the prevention of secondary cardiovascular events in patients with documented CAD [162-168] and showed higher intake of n-3 PUFAs lowers the number of mortalities related to cardiovascular diseases (CVD) [169-174]. For example, in a prospective cohort study, Mozaffarian et al. demonstrated higher plasma levels of n-3 PUFA were associated with lower total mortality rates with fewer cardiovascular compared to non-cardiovascular deaths in older adults [175]. In contrast, recent clinical studies challenge the cardiovascular benefits of n-3 PUFAs, indicating a weak or even non-significant relationship between omega-3 fatty acids and reduction in cardiovascular risk, and thus raise questions about the cardiovascular benefits of n-3 PUFAs [176]. Three double-blind trials, the Alpha Omega, the OMEGA and the SU.FOL.OM3, failed to show any additional benefit of n-3 PUFAs on major cardiovascular endpoints [177-179] as well as recent studies have yielded non-significant or less promising results for the cardioprotective effects of n-3 PUFAs [177, 178, 180, 181]. The discrepancies in clinical findings between beneficial and non-beneficial effects might be attributed to several factors, such as overall improved cardiovascular therapy, which includes increased use of beta-blockers, ACEis or ARBs masking any benefits of n-3 PUFAs in more recent studies. Moreover, a lack of standardization of treatment doses, drug formulations and dietary supplementation in the studies impacts bioavailability and cardiovascular effects. For example, marketed products containing the ethyl ester formulation of

n-3 PUFAs have been shown to have reduced bioavailability compared to the free fatty acids [182-184].

Overall, there remains uncertainty regarding the beneficial effects n-3 PUFAs toward cardiovascular events and mortality rates (Table 1 and 2). However, despite these conflicting data, the consumption of n-3 PUFA is recommended by the American Heart Association (AHA) to prevent clinical CVD events in individuals with prevalent CHD, such as a recent MI, to reduce mortality rates and individuals with prevalent HF without preserved left ventricular function to reduce hospitalizations and number of deaths [185, 186]. Importantly, there is a growing understanding of how different metabolites generated from n-3 PUFA impact cellular and organ function, which is providing insight into their potential beneficial role in cardiovascular health [187, 188].

The cardiovascular benefits of n-3 PUFAs may be attributed to their pleiotropic effects on the different components of the cardiovascular system, such as the enrichment of membranes leading to improved organelle and cellular function [189], autonomic tone [190, 191], increasing arrhythmic thresholds [192] and reducing blood pressure [191, 193]. Increased consumption of n-3 PUFAs has a favorable effect on lipid profiles as they replace saturated fatty acids and lower blood triglyceride levels which can stabilize atherosclerotic plaques protecting against IHD [57, 194]. Supplementation with EPA and DHA could also exert a protective effect on the heart through enriching mitochondrial membrane phospholipids composition and thus improving mitochondrial function and increasing the efficiency of ATP generation [195, 196]. Additional cardiovascular benefits of n-3 PUFA stem from their diverse anti-inflammatory properties [197]. For example, the ability of n-3 PUFAs to blunt the excessive activation of the innate immune system was demonstrated to have a positive cardiovascular impact abrogating the progression of IHD [198, 199]. The anti-inflammatory effects of n-3 PUFAs may reduce and stabilize atherosclerotic lesions, which can potentially lead to better outcomes in CAD [200-202].

In addition, growing evidence demonstrates the ability of n-3 PUFAs to reduce circulating levels of inflammatory cytokines, chemokines and pro-inflammatory AA-derived metabolites [195, 203, 204]. The anti-inflammatory mechanisms of n-3 PUFAs and their metabolites have an important role in regulating and protecting cardiovascular function (Table 3).

6. Mechanisms of n-3 PUFAs to reduce inflammation and protect against IHD

Several basic, clinical and epidemiological studies hypothesize that the cardioprotective effects of n-3 PUFAs against IHD are attributed mainly to their immunomodulatory properties [198, 205-208]. The anti-inflammatory effects of EPA, DHA and their biologically active metabolites, are mediated mainly by G-protein coupled receptors (GPR), particularly GPR120 [209], and nuclear receptors particularly peroxisome proliferator-activated receptors (PPAR)- α/γ [210]. This section will highlight the immunomodulatory mechanisms of n-3 PUFAs and the associated cardioprotection against IHD.

6.1. Metabolite-independent effects

6.1.1 Modulation of gene expression of different innate immune components

N-3 PUFAs can regulate the transcription and the expression of inflammatory genes including cytokines, chemokines and adhesion molecules in cardiomyocytes, fibroblasts, endothelial cells, monocytes and macrophages [211-215]. N-3 PUFAs alter the expression of these genes through regulating transcription factors, such as the blocking the action of the pro-inflammatory NF- κ B [216-219], and activating the anti-inflammatory transcription factors PPAR α/γ [210, 220]. Activation of PPAR α/γ transcription factors is believed to directly interfere with the activation of NF- κ B and prevent its translocation to the nucleus reducing the inflammatory burst [221-223]. Mishra et al., demonstrated anti-inflammatory properties of fish oil may result from the inhibitory effects of EPA and DHA on NF- κ B activation via a PPAR α -dependent pathway in both human umbilical vein endothelial cells (HUVEC) and microvessel endothelial cells [224]. This is supported by evidence from data showing treatment of

differentiated THP-1 and HUVECs with EPA led to the upregulation PPAR α which inhibited NF- κ B activation and attenuated TNF α -induced production of MMPs [201]. Another important immunomodulatory mechanism involves activating the GPR120 receptor, which mediates robust and broad anti-inflammatory effects. Research from Oh et al., indicated n-3 PUFAs act on and stimulate GPR120 in both monocytic RAW 264.7 cells and primary intraperitoneal macrophages inhibiting TLR4-mediated inflammatory responses blocking NF- κ B activation. Knockdown of GPR120 knockdown attenuated the protective effects attributed to n-3 PUFA consumption [209] (Fig.4).

Incorporation of n-3 PUFA such as EPA directly into human atherosclerotic plaques has been associated with a reduced number of foam cells and T cells, less inflammation and increased plaque stability. While the exact mechanism was unknown, the beneficial effects were attributed to suppression of extracellular matrix proteins MMP-7, MMP-9 and MMP-12 involved in remodeling [225]. Limiting adverse left ventricular remodeling and myocardial fibrosis caused by MI or pressure overload stems from an ability of n-3 PUFA to regulate fibrosis and inflammatory signaling. Evidence demonstrates inhibiting the TGF- β 1-induced smad2/3 pathway or activating GPR120 signaling to regulate TAK1 and downstream NF- κ B responses are potential mechanisms [209, 226]. Eclov et al. demonstrated EPA, but not DHA, prevented cardiac fibrosis in a mouse model of pressure overload-induced HF via activation of GPR120 and blocking the TGF- β fibrotic pathway [227]. Long-term administration of EPA in mice for 28 days before and 28 days after experimental MI induction improved the prognosis, reduced the post-MI fibrosis and limited LV remodeling via inhibition of the TGF- β /Smad signaling and promoting macrophage polarization toward the anti-inflammatory M2 phenotype [228]. Further evidence, demonstrated oral administration of EPA to DahlS.Z-Leprfa/Leprfa (DS/obese) rats increased adiponectin secretion which inactivated NF- κ B signaling leading to a reduction in cardiac fibrosis and attenuation of diastolic dysfunction [229].

6.1.2 Altering the cell membrane structure

Incorporation of n-3 PUFAs into membrane phospholipid bilayers proposes potential insight into the immunomodulatory effects by altering membrane architecture and protein function, which impacts membrane-mediated signaling, generation of bioactive lipids, gene activation, protein trafficking and cytokine secretion [230-234]. The increased membrane incorporation may alter both innate and adaptive immune responses, including the maturation of dendritic cell, macrophage function, as well as T and B cell polarization/activation [235-240]. It was demonstrated that DHA was better than EPA in replacing n-6 PUFAs and cholesterol in plasma membranes of aortic endothelial cells increasing the fluidity of the phospholipid membrane [241]. A change in fluidity can interfere with membrane protein, receptor and transporter function such as the dimerization and expression of the TLR4 subunits, impeding the downstream inflammatory response [242, 243]. Inflammatory cells such as neutrophils, monocytes, macrophages and lymphocytes often contain a large proportion of AA in their membrane. The activation of phospholipase A₂ (PLA₂), the enzyme that liberates AA from the cell membrane, is amongst the earliest biochemical alterations in ischemic myocardium [244-246]. Free AA becomes afterwards a potent source of pro-inflammatory metabolites. However, the substitution of AA with EPA and DHA in the cell membrane, by increasing the consumption of n-3 PUFAs, can alter immune cell reaction in response to inflammatory stimuli by shifting the metabolic profile to less proinflammatory or even anti-inflammatory metabolic profile [247-250].

6.2. Metabolite-dependent effects

As illustrated earlier, the metabolism of n-3 and n-6 PUFAs is closely intertwined as their metabolic pathways compete for the same enzymes. In most cell types, AA is the prevalent PUFA present in membrane phospholipids of cells; for example, in mononuclear cells taken from healthy volunteers consuming a typical Western diet, the mean proportions of LA, DGLA, AA, EPA and DHA were 10, 2, 20, 0.5 and 2.5% of the total fatty acid content [251]. Liberation

of AA from the cell membrane by activated PLA2 under stress conditions generates a wide variety of pro-inflammatory metabolites [252]. The released AA acts as a substrate for cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) enzymes to yield a family of oxygenated metabolites. COX converts AA to the 2-series of prostaglandins (PGs) and the 2-series of thromboxanes (TxA), while LOX enzymes metabolize AA to the 4-series LTs and the hydroxyicosatetraenoic acids (HETEs) (Fig. 3). These metabolites are considered pro-inflammatory mediators that are involved in various pathological processes including IHD [253-256]. Following consumption, n-3 PUFAs compete with n-6 PUFAs for incorporation into cell membranes and for active sites in COX and LOX enzymes to produce less potent pro-inflammatory or even anti-inflammatory metabolites [257]. For example, the production of PGE2 and LTB4 by human inflammatory cells was significantly decreased in a diet rich in fish oil [258-261]. N-3 PUFAs can act as a substrate for COX and 5-LOX enzymes resulting in production of the 3-series of PGs and Txs as well as the 5-series LTs, which are a set of less inflammatory or even anti-inflammatory metabolites in comparison to the metabolite family derived from AA [262, 263]. Importantly, 5-, 12- and 15-LOX enzymes are involved in the formation of potent anti-inflammatory mediators derived from the metabolism DHA and EPA called resolvins, protectins and maresins (Fig.2). Therefore, the metabolism of n-3 PUFAs by COX and LOX enzymes reduces AA-derived pro-inflammatory metabolites and shifts the metabolic profile toward anti-inflammatory mediators, suggesting a central cardioprotective mechanisms of n-3 PUFAs.

6.2.1 COX-derived metabolites of n-3 PUFAs

Numerous studies have shown COX-mediated metabolites of n-6 PUFAs, 2-series PGs and 2-series TxA, play an important role in the pathogenesis of CAD. For instance, TxA2, a potent vasoconstrictor and platelet aggregator, participates in the initiation and progression of atherogenesis through induction of leukocyte-endothelial cell interaction, platelet activation and thus thrombus formation after the rupture of the atherosclerotic plaque [264]. In addition, a

predominant metabolite found in macrophages, PGE₂, induces the expression of MMP enzymes which are crucial in the degradation of atherosclerotic plaque, triggering thrombosis and thus MI induction [265]. However, evidence indicates PGE₂ or its analogues can also protect the heart from IR injury via activation of its receptor subtype E prostanoid receptor 4 (EP₄), suggesting that EP₄ agonists are probably useful for protection against reperfusion-induced cardiac injury [266]. Moreover, Degousee et al. demonstrated PGE₂ could impart a beneficial effect in the infarcted heart by preventing the pathological myocardial remodeling and improve cardiac function after MI [267, 268].

Experimental evidence demonstrate diets rich in n-3 PUFAs shift the balance from TxA₂ to TxA₃ production, increase the levels of PGI₃ and PGE₃ and decrease COX-2 gene expression, reducing the pro-inflammatory mediators and effects attributed to metabolism of AA [269-273]. For example, TxA₃ possesses significantly less potent platelet activation and vasoconstriction properties making it less pro-thrombotic than TxA₂ [274, 275]. Moreover, n-3 PUFAs decrease the affinity of the TxA₂ receptor for TxA₂, thus inhibiting TxA₂-induced platelet aggregation [276]. Research from Tull et al. demonstrated EPA-derived metabolites, PGD₃, can antagonize neutrophil recruitment induced by the AA metabolite, PGD₂, reducing the inflammatory response [277]. 18-hydroxyeicosapentaenoic acids (18-HEPE) is another important metabolite produced from the metabolism of EPA via either aspirin-acetylated COX-2 [278] or cytochrome P450 monooxygenase [279] enzymes, which possess important anti-inflammatory and anti-fibrotic properties. For example, 18-HEPE was able to prevent macrophage infiltration, cardiac fibrosis and remodeling in a model of transverse aortic constriction (TAC) pressure overload [280]. Together, these studies highlight the complex nature of COX metabolites in the cardiovascular system.

6.2.2 LOX-derived metabolites of n-3 PUFAs

When n-6 PUFAs predominate in cell membranes, proinflammatory mediators such as LTs are produced via the LOX pathways. Conversely, higher ratios of n-3 PUFAs promote secretion of less potent LTs, resulting in a shift to a milieu of less inflammatory mediators. LOX enzymes catalyze the oxidation of AA to produce hydroperoxyeicosatetraenoic acids (HpETEs), which are then reduced to form their HETE derivatives. 5-LOX, 12-LOX and 15-LOX catalyze the metabolism of AA to 5-HETE, 12-HETE and 15-HETE, respectively, which are present in the heart [281-284]. LOX enzymes have a higher affinity for n-3 PUFAs and increased consumption of n-3 PUFAs favors the production of the less pro-inflammatory LTs than the AA-derived inflammatory mediators. For instance, Chapkin et al. illustrated the increased generation of 5-series LTs in macrophages of fish oil-fed mice [272] and neutrophils from humans supplemented with oral fish oil for several weeks [258, 285-288]. Therefore, an increased availability of n-3 PUFAs can shift the metabolism from the detrimental LOX-mediated metabolites of AA, to the less biologically active n-3 PUFA-derived LTB₅ metabolite which possesses 10 to 100 times reduced potency [263, 289-293].

LOX-mediated HETEs are pro-inflammatory and tend to be produced excessively in models of myocardial IR injury [294]. 5-HETE and 12-HETE levels were found to increase significantly in cultured canine myocytes following hypoxia reoxygenation conditions [295]. HETEs play a significant role in the recruitment of leucocytes to damaged areas, production of pro-inflammatory cytokines and contribute to non-resolving inflammation in cardiac pathology [296, 297]. Indeed, elevated expression of 12/15-LOX in mice results in increased pro-inflammatory markers such as MCP-1 and IL-6 and recruitment of monocytes, as well promotes monocyte–endothelial cell interactions that lead to atherogenesis [298-300]. Whereas, 12/15-LOX null mice have significantly lower potential to develop atherosclerosis [301]. Interestingly, reduced plasma levels of 12-HETE in 12/15-LOX null mice resulted in better post-MI survival secondary to the advanced resolution of inflammation [302]. Metabolite products of 12-LOX

have a role in the development of cardiac fibrosis and hypertrophy, for example, overexpression of 12-LOX in rat fetal cardiac fibroblasts resulted in growth of cardiac fibroblasts associated with significant elevation of collagen and fibronectin levels, indicative of a fibrotic phenotype [303, 304].

The 5-LOX enzyme catalyzes the conversion of AA to LTA₄, an unstable intermediate, which can be metabolized by LTA₄ hydrolase to LTB₄, a potent chemoattractant, or conjugated to glutathione producing the cysteinyl LTs (CysLTs), including LTC₄, LTD₄, and LTE₄ [305-307]. Interest in the possible involvement of LTs in the development of IHD stems from studies demonstrating robust relationships between LTs and an increased risk of atherosclerotic plaques and development of MI [308, 309]. The 4 series LTs from 5-LOX mediated metabolism are abundantly expressed in arterial walls of patients with atherosclerosis. As 5-LOX is mainly localized in macrophages, dendritic cells, foam cells, mast cells and neutrophilic granulocytes, increased numbers of these cells, and consequently LT production, are associated with atherosclerotic lesions [310-312]. LTs have a role in the migration and infiltration of leukocytes to injured tissues as several reports indicate a correlation between myocardial infarct size with the extent of LT-mediated leukocyte recruitment to the injured myocardium [86, 313].

Involvement of CysLT in the pathogenesis and progression of IHD comes from studies demonstrating increases in LTC₄ and LTD₄-mediated expression of the adhesion molecule P-selectin in human endothelial cells and enhanced pro-inflammatory signals of IL-8, CXCL-2 and COX-2 correlating with worse outcomes [314-316]. Earlier studies demonstrated increased levels of CysLTs in CAD had numerous aggravating consequences including vasoconstrictive effects on coronary arteries, inducing coronary smooth muscle cell proliferation and inflammation as well as negative inotropic action all of which worsen the prognosis [317, 318]. Ni et al. showed that activation of endothelial and non-endothelial CysLT receptors increases vascular permeability and facilitates the recruitment of leukocytes exacerbating the

consequences of IR injury [319]. While the exact mechanisms remain unknown, early work by Hock et al. showed that antagonism of CysLTs receptor reduced the magnitude of myocardial necrosis in a feline model of IR injury [320]. Together, the detrimental effects of CysLT may worsen the clinical manifestation of IHD.

6.2.3 CYP-derived metabolites of n-3 PUFAs

CYP2J and CYP2C isoforms, the constitutively expressed CYP epoxygenases found in the cardiovascular system metabolize EPA into 5 regioisomeric epoxyeicosatetraenoic acids (5,6-, 8,9-, 11,12-, 14,15-, 17,18-EEQ) and DHA into 6 regioisomeric epoxydocosapentaenoic acids (4,5-, 7,8-, 10,11-, 13,14-, 16,17-, 19,20-EDP) [321-325]. CYP epoxygenases preferentially catalyze the epoxidation of the terminal double bond of n-3 PUFAs generating 17,18-EEQ and 19,20-EDP which become the predominant endogenous lipid mediators produced in most tissues, including the lung, kidney, heart and plasma [323, 326, 327]. Of note, these epoxy lipids appear to be more effective at lower concentrations compared to their parental n-3 PUFA. For example, Falck et al., showed that 17,18-EEQ was able to protect neonatal rat cardiomyocytes against Ca^{2+} -overload with an EC_{50} of 1–2 nM, while EPA required prolonged incubation and a ~1000-fold higher concentration to produce the same effect [328]. The epoxy metabolites EEQs and EDPs may then undergo further metabolism by soluble epoxide hydrolase (sEH) enzymes to corresponding inactive diols [329, 330]. Because the ω -3 double bond distinguishing EPA and DHA from AA is the preferred site of attack by human CYPs, n-3 PUFAs compete with AA as alternate substrates for CYP metabolism. Accordingly, supplementation with EPA and DHA increases the proportion of EEQ and EDP metabolites at the expense of the AA-derived CYP epoxy metabolite, epoxyeicosatrienoic acids (EET) [323].

Recent evidence indicates the CYP-derived metabolites, 17,18-EEQ and 19,20-EDP, are responsible for mediating different anti-inflammatory effects of n-3 PUFAs in various models of injury [323, 331-333]. Fang et al., demonstrated a n-3 PUFA-rich diet given to mice

attenuated MI injury by shifting the metabolite profile to more anti-inflammatory mediators, increasing 19,20-EDP and 17,18-EEQ levels while decreasing PGE2 [334]. In response to cardiac IR injury, the innate immune system triggers inflammatory reactions resulting in both protective and detrimental outcomes, which involves NLRP3 inflammasomes and proinflammatory cytokines. In a mouse model of IR injury, DHA and 19,20-EDP exerted cardioprotective properties resulting in improved postischemic functional recovery associated with attenuation of NLRP3 inflammasome complex activation and preserved mitochondrial function [82]. Interestingly, the attenuation of NLRP3 inflammasome activation was not observed following treatment with EPA or 17,18-EEQ, and importantly inhibition of CYP epoxygenase activity prevented the conversion of DHA to 19,20-EDP abolishing the protective effect [82]. Anti-inflammatory effects of CYP-derived epoxy metabolites have been demonstrated in other conditions, such as 19,20-EDP inhibited TNF α -induced retinal vascular inflammation and intraperitoneal infusions of 17,18-EEQ and 19,20-EDP protected against allergic intestinal inflammation and kidney fibrosis in respective mouse models [335, 336]. 17,18-EEQ inhibited TNF- α -induced inflammation in human bronchi via repression of NF- κ B and activation of the transcription factor PPAR- γ , in which the action of 17,18-EEQ was enhanced by sEH inhibition [337]. Using an animal model of inflammatory pain, Morisseau et al. demonstrated the DHA epoxides, but neither the parent fatty acid nor the corresponding diols, selectively modulate nociceptive pathophysiology [330]. The bacterial endotoxin, lipopolysaccharide (LPS) has a significant role in causing numerous cardiovascular complications involving adverse inflammatory effects. Recently, it was demonstrated that 19,20-EDP protected HL-1 cardiac cells from LPS-stimulated inflammatory cell injury by preserving mitochondrial integrity and biogenesis [338]. Although the precise molecular mechanisms remain unknown, 19,20-EDP-mediated effects activated SIRT1 signaling to promote mitobiogenesis and attenuate NF- κ B activity [338]. Together, accumulating evidence suggests the anti-inflammatory properties of CYP-epoxygenase metabolites of n-3 PUFAs provide

important protective responses in models of cardiovascular injury. However further investigation is required to elucidate their mechanisms and the extent to which they are involved in cardioprotection.

AA can be metabolized by CYP ω -hydroxylases into mid-chain 5-, 8-, 9, 11-, 12- and 15-HETEs, terminal 20-HETE as well as subterminal 19-, 18-, 17- and 16-HETEs (Fig. 3) [339, 340]. The ability of mid-chain and terminal HETEs to induce inflammatory responses form part of the basis for their detrimental effects toward IHD and in the development of CVD [341-344]. Kayama et al. showed 12-HETE has a role in the development of HF by increasing MCP-1 expression in cardiac fibroblasts and endothelial cells as well as increasing the infiltration of macrophages into the myocardium leading to cardiac fibrosis [345]. In addition, Maayah et al., demonstrated that 12-HETE, 15-HETE as well as 5-HETE are potent inducers of NF- κ B activation in RL-14 cells, a human ventricular cardiomyocytes cell line [346]. Similarly, 20-HETE activates NF- κ B signaling and induces expression of cellular ICAM-1 adhesion molecules, thereby promoting inflammation leading to vascular endothelial dysfunction, an important component in the pathogenesis of IHD diseases [347]. Coronary plasma concentrations of 20-HETE are markedly increased during ischemia and following reperfusion contributing to infarct size development. Accordingly, the selective inhibitors of CYP4A, the main 20-HETE-forming ω -hydroxylase, reduce ischemic infarct size in IR injury in canine myocardium [348-350]. Consistent with animal studies, the role of HETEs in aggravating CHD has been correlated with human data indicating concentration of HETEs is markedly higher in symptomatic atherosclerotic plaques, as compared with asymptomatic ones [351, 352]. Importantly, while there is limited data available regarding n-3 PUFAs, CYP ω -hydroxylases preferentially metabolize EPA into hydroxyeicosapentaenoic acids (19- and 20- HEPE) and DHA into hydroxydocosahexaenoic acids (21- and 22-HDoHE) at the expense of AA- derived HETEs, which are thought to possess anti-inflammatory properties [330, 353-355]. Therefore, increasing

consumption of n-3 PUFAs will cause a decrease in the levels of the CYP hydroxylase-derived pro-inflammatory metabolites with a concomitant increase in EPA- and DHA-derived anti-inflammatory metabolites which will have a beneficial impact on the cardiovascular health.

6.2.4 *Resolvins: The anti-inflammatory and resolving mediators*

Important lipid mediators involved in regulating inflammatory responses generated from the metabolism n-3 PUFAs include resolvins 'resolution phase interaction products' produced from both EPA (E-series, RvE1-2) and DHA (D-series, RvD1-6) as well as protectins and maresins produced from DHA [278, 356, 357]. The synthesis of resolvins, protectins and maresins involve both the COX and LOX pathways, with different epimers being produced in the presence and absence of aspirin [358-361]. Resolvins and protectins, produced from EPA and DHA, were first discovered in inflammatory exudates during the acute inflammatory process indicating their role in the inflammation [278, 356, 362]. Several studies demonstrated resolvins, protectins and maresins possess potent anti-inflammatory and inflammation resolving properties indicating an importance in terminating ongoing inflammatory processes. These unique metabolites promote the resolution of acute inflammation by preventing the migration of neutrophils and monocytes across epithelial cells and promoting clearance of PMNs, apoptotic cells, and debris from the site of inflammation [356, 363]. For example, Krishnamoorthy et al. showed that resolvins inhibit neutrophil tissue infiltration by decreasing the production of the chemokine IL-8 and reducing the expression of surface adhesion receptors on the neutrophils, such as CD11b or CD18 [364]. Resolvins also reduced neutrophil-derived ROS production, favored neutrophils apoptosis and clearance by macrophages, as well as participated in shutting off chemokine signaling [365-367]. The partial agonist/antagonist activity of RvE1 on the LTB₄ receptor on PMNs serves to inhibit NF- κ B activation, abolish pro-inflammatory cytokines production and reduce PMN infiltration [356, 357, 368]. Very recently, Sulciner et al., showed that RvD1, RvD2, or RvE1 inhibits debris-stimulated cancer progression by enhancing

clearance of debris via macrophage phagocytosis in multiple tumor types. These resolvins suppressed the release of the proinflammatory cytokines/chemokines, including TNF α , IL-6, IL-8, CCL4, and CCL5, by human macrophages cocultured with tumor cell debris [369]. It is believed that E and D-resolvins present a similar function; both can inhibit NF- κ B by a mechanism which is PPAR- γ dependent and mediate most of their actions via specific G-protein coupled receptors [272, 370, 371].

Since inflammation has a direct role in the pathogenesis of CVD, particularly IHD, resolvins, due to their anti-inflammatory properties, can improve the prognosis. For example, Morin et al. demonstrated a diet enriched with DHA and monoglycerides significantly increased the levels of RvD2 and RvD3, which correlated with reduced levels of proinflammatory mediators C-reactive protein (CRP), IL-6, TNF- α , and IL-1 β in a rat model of hypertension [372]. Several experimental studies illustrated the ability of resolvins to significantly reduce atherosclerotic lesions, as observed in mouse models that lack LOX-12 and LOX-15, the two enzymes responsible of resolvins synthesis, which have accelerated atherosclerosis development [373]. Furthermore, Viola et al. showed administration of RvD2 and Maresin 1 in a mouse model of atherosclerosis induces changes in the macrophage profile from an inflammatory (M1) toward a reparative phenotype (M2) which contributes to plaque stability and thus prevents atheroprogession [374]. Consumption of n-3 PUFAs by patients suffering from CAD were able to restore the levels of pro-resolving lipid mediators and promote macrophage phagocytosis of blood clots *in vitro* [375]. Moreover, RvE1 administration was demonstrated to reduce TNF- α and interferon- γ (IFN- γ) gene expression in aorta, decrease the levels of the inflammatory marker CRP as well as reduce macrophage infiltration into intima and thus attenuate atherosclerosis and atherosclerotic plaque formation [376-378]. As a consequence of atherosclerosis, VSMCs become more proliferative, chemotactic and have an enhanced production capacity of pro-inflammatory cytokines [379]. Evidence demonstrates resolvins are

capable of reducing the VSMCs responses via local activation of resolution mechanisms and abolishing leukocyte recruitment thereby ameliorating the atheroprogession [380, 381]. Consistent with these effects, oxidative stress levels and NF- κ B activation were significantly lower in the RvD1-treated VSMC samples [382, 383].

The role of resolvins in protection against cardiac ischemia and reperfusion injury has been documented to involve numerous mechanisms including anti-inflammatory properties. Both *in vivo* and *in vitro* models of IR injury have demonstrated RvE1 can reduce infarct size, decrease apoptosis and improve cardiomyocyte survival [384]. Similar results were reported for the cardioprotective effects of RvD1, where RvD1 diminished infarct size and neutrophil accumulation in the infarcted myocardium and decreased post-myocardial infarct depression [385-387]. RvD1 alleviates post-MI inflammation by limiting neutrophil recruitment in the spleen and LV, increasing resolving lipid mediators, altering the macrophages phenotype post-MI and reducing the expression of pro-fibrotic genes and collagen deposition. Together, these results indicated that RvD1 can modulate the pathophysiology of resolution in order to limit cardiac remodeling and thus prevent the progression of HF following MI [388].

7. Summary and conclusion

In this review, we highlighted the detrimental role of the uncontrolled activation of the innate immune system in worsening the clinical outcomes associated with IHD. We focused on immunomodulatory properties of n-3 PUFAs and their bioactive metabolites, illustrating their potential cardioprotective effects. Dietary or non-dietary intake of n-3 PUFAs and/or their biologically active metabolites have an ability to inhibit many of the adverse effects of an immune response. Accordingly, increasing amount of studies demonstrate the ability of n-3 PUFAs to mitigate the negative consequences of IHD including atherosclerosis, MI, IR injury and cardiac remodeling. Several mechanisms contribute to the immunomodulatory effects of n-3 PUFA including altering cell membranes composition, modifying both cell signaling and gene

expression, shifting the pattern of the lipid metabolites produced under stress conditions to a more anti-inflammatory metabolite profile. Despite the promising immunomodulatory effects of n-3 PUFAs, more clinical and epidemiological research is warranted to translate these results.

Conflicts of interest

The authors have no conflicts to declare

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Figure legends

Figure 1: Schematic diagram showing the inflammatory response to acute injury. MI induced injury to cardiomyocytes triggers a pro-inflammatory response through the production of DAMPS and ROS, which act on the PRR (TLR, NLR) on the nearby cardiomyocytes, endothelial cells, fibroblasts and resident immune cells. Activation of these receptors stimulate the release of several cytokines and chemokines (such as IL-1 β , IL-18, IL-1 α , IL-6, TNF- α , CCL2, CCL5), which mediate the recruitment and infiltration of inflammatory immune cells (neutrophils, monocytes and macrophages) from the peripheral blood stream, spleen and bone marrow to the injured myocardium. The migration of these cells aggravates the myocardial injury through releasing of additional pro-inflammatory cytokines. DAMPs, Damage-associated molecular

patterns; IR: Ischemia-Reperfusion; ROS, Reactive oxygen species; mtDNA, mitochondrial DNA; NLRP3, NACHT, LRR and PYD domains-containing protein 3; NLR: NOD-like receptor; PRRs, Pattern recognition receptors; TLRs, Toll-like receptors.

Figure 2: Overview of n-3 PUFA Metabolism. ALA, EPA and DHA are essential fatty acids obtained from dietary sources. EPA and DHA found in cell membrane phospholipids can be released by the enzyme PLA₂. Subsequently, EPA and DHA can be metabolized by COX, LOX and CYP enzymes into a vast array of differing metabolites with numerous physiological functions. ALA, α -Linolenic acid; COX, Cyclooxygenase; CYP, Cytochrome P450; DHA, Docosahexaenoic acid; DiHDPa, Dihydroxydocosapentaenoic acid; DHEQ, Dihydroxyeicosatetraenoic acid; EDP, Epoxydocosapentaenoic acid; EEQ, Epoxyeicosatetraenoic acid; EPA: Eicosapentaenoic acid; HEPE, Hydroxyeicosapentaenoic acid; HpDHA, Hydroperoxydocosahexaenoic acid; LT, Leukotriene; LOX, Lipoxygenase; MaR, Maresin; PD, Protectin; PG, Prostaglandin; PLA₂, Phospholipase A₂; PUFA, Polyunsaturated fatty acid; Rv, Resolvin; sEH, Soluble epoxide hydrolase; Tx, Thromboxane.

Figure 3: Overview of n-6 PUFA Metabolism. LA and AA are essential fatty acids obtained from dietary sources. AA found in cell membrane phospholipids can be released by the enzyme PLA₂. Subsequently, AA can be metabolized by COX, LOX and CYP enzymes into a vast array of differing metabolites with numerous physiological functions. AA, Arachidonic acid; COX, Cyclooxygenase; CYP, Cytochrome p450; CysLTs, Cysteinyl leukotrienes; DHET, Dihydroxyeicosatrienoic acid; DGLA, Dihomo- γ -linolenic acid; EET, Epoxyeicosatrienoic acids; GSH, Glutathione; HETE, Hydroperoxyeicosatetraenoic acid; HpETE, Hydroperoxyeicosatetraenoic acid; LA, Linoleic acid; LOX, Lipoxygenase; LT, Leukotriene; PG, Prostaglandin; PLA₂, Phospholipase A₂; PUFA, Polyunsaturated fatty acid; sEH, Soluble epoxide hydrolase; Tx, Thromboxane.

Figure 4: Schematic diagram of n-3 PUFAs immunomodulatory effects. The anti-inflammatory and anti-fibrotic effects of n-3 PUFAs and their metabolites are ascribed to their ability to: (1) incorporate into the cell membrane and displace AA as an alternative substrate for PLA₂, (2) alter the lipid raft restricting the dimerization and pro-inflammatory signaling of TLR, (3) activate GPCR mediated signaling that stimulates PPARs and inhibits NF-κB activity, (4) undergo CYP epoxygenase mediated metabolism into the corresponding anti-inflammatory oxylipins, (5) inhibit the NLRP3 inflammasome cascade, (6) prevent the activation of the pro-fibrotic TGF-β signaling pathway, and, (7) undergo metabolism into anti-inflammatory and pro-resolving lipid mediators resolvins, protectins, and maresins. AA, Arachidonic acid; COX, Cyclooxygenase; CYP, Cytochrome p450; DHA, Docosahexaenoic acid; DAMPs, Damage-associated molecular patterns; EPA, Eicosapentaenoic acid; GPCR, G-protein coupled receptor; IL, Interleukin; LOX, Lipoxygenase; MI, Myocardial infarction; NF-κB, Nuclear factor kappa-light-chain enhancer activated B-cells; NLRP3, NACHT, LRR and PYD domains-containing protein 3; PLA₂, Phospholipase A₂; PPAR, Peroxisome proliferator-activated receptor; TLR, Toll- like receptor; TGF, Transforming growth factor.

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Table 1: Clinical trials showing positive effect of n-3 PUFAs in the cascade of IHD

Sample criteria and size (n)	Treatment Protocol	Key Findings	Conclusion	Reference
<ul style="list-style-type: none"> - DART Trial: - Prior MI - < 70 years old - (2033) 	<ul style="list-style-type: none"> - Advised to consume 2-3 weekly portions (200-400g) of fatty fish vs. no advice - 2 years 	<ul style="list-style-type: none"> - Significantly reduced death rate (RR=0.71) and reduced IHD event (RR=0.84). 	<ul style="list-style-type: none"> - 2-3 servings of fatty fish per week may reduce all cause mortality and IHD-related deaths in male patients with a history of MI. 	[164]
<ul style="list-style-type: none"> - GISSI – Prevenzione Trial: - Recent MI (≤ 3 months) - (11, 324) 	<ul style="list-style-type: none"> - 0.85-0.882g/day EPA and DHA ethyl esters vs. no treatment control - 3.5 years 	<ul style="list-style-type: none"> - Significantly reduced death, non-fatal MI or stroke (RR=0.85). 	<ul style="list-style-type: none"> - N-3 PUFA supplementation may be effective for secondary prevention of CV events and death. 	[389]
<ul style="list-style-type: none"> - Patients awaiting carotid endarterectomy - (188) 	<ul style="list-style-type: none"> - Fish oil capsules (0.86g/day EPA and 0.52g/day DHA) vs. sunflower oil capsule or placebo control - 42 days 	<ul style="list-style-type: none"> - Significantly higher EPA and DHA in lipid fractions of plaques. - Significantly reduced plaque macrophage infiltration. - No difference in ICAM-1 or VCAM-1 levels and T lymphocytes in plaques. 	<ul style="list-style-type: none"> - N-3 PUFA supplementation may increase carotid plaque stability via reduction of thinning of fibrous caps and plaque inflammation. 	[194]
<ul style="list-style-type: none"> - Autopsy results of deceased Alaskan Natives and Non-natives - Unmatched - (245) 	<ul style="list-style-type: none"> - Observational study. - No intervention 	<ul style="list-style-type: none"> - Significantly higher proportion of EPA and DHA in adipose tissue TG. - Significantly fewer raised atherosclerotic lesions in abdominal LAD coronary artery and right coronary Alaskan Natives. 	<ul style="list-style-type: none"> - Higher dietary intake of n-3 PUFAs may correlate with the observed increase in n-3/n-6 PUFA ratio in adipose tissue and reduced severity of coronary artery plaques. 	[390]
<ul style="list-style-type: none"> - Postmenopausal women - Established CAD - Previous coronary angiography - (288) 	<ul style="list-style-type: none"> - Observational prospective cohort study. - No intervention - 3.2 years 	<ul style="list-style-type: none"> - Significantly reduced mean coronary artery diameter, stenosis, and new lesion development in patients with DHA levels above median values. 	<ul style="list-style-type: none"> - Higher plasma DHA levels may be associated with reduced coronary plaque progression in post-menopausal women with a history of CAD. 	[167]

<ul style="list-style-type: none"> - Confirmed CVD and/or RA and/or OA - Elevated CRP - (99) 	<ul style="list-style-type: none"> - Neptune Krill Oil (NKO) 300mg daily (17% EPA and 10% DHA) vs. placebo - 30 days 	<ul style="list-style-type: none"> - Significantly reduced CRP levels by 7, 14, and 30 days. 	<ul style="list-style-type: none"> - Short term EPA/DHA supplementation may reduce systemic inflammatory response in various chronic inflammatory pathologies and improve overt clinical symptoms. 	[391]
<ul style="list-style-type: none"> - T2DM - Metabolic syndrome - (44) 	<ul style="list-style-type: none"> - 1.8g/day EPA (>98% EPA ethyl ester) + diet intervention vs. diet alone - 3 months 	<ul style="list-style-type: none"> - Significantly reduced CRP, sdLDL, CETP activity, and RLP-TG from baseline. 	<ul style="list-style-type: none"> - EPA supplementation may reduce markers of inflammatory response and improve serum lipid profile in patients suffering from metabolic syndrome at risk for CVD. 	[392]
<ul style="list-style-type: none"> - JELIS Trial: - TC \geq 6.5 mmol/L - LDL-C \geq 4.4mmol/L - Statin treatment - (18,645) 	<ul style="list-style-type: none"> - 1.8g/day EPA vs. statin treatment only - 4.6 years 	<ul style="list-style-type: none"> - Significantly reduced major coronary events (HR=0.81) and unstable angina (HR=0.76). - Reduced fatal and non-fatal MI, coronary events and death. 	<ul style="list-style-type: none"> - Combined EPA and statin therapy in patients with dyslipidemia may reduce the incidence of major coronary events. 	[393]
<ul style="list-style-type: none"> - GISSI-HF Trial: - Chronic heart failure - NYHA class II-IV - (6975) 	<ul style="list-style-type: none"> - 0.85-0.882g/day EPA and DHA ethyl esters vs. placebo - 3.9 years 	<ul style="list-style-type: none"> - Significantly reduced time to death (HR=0.91) and combined time to death or - CV hospital admission (HR=0.92). 	<ul style="list-style-type: none"> - Patients with chronic heart failure supplemented with n-3 PUFAs may experience prolonged survival and reduced CV-associated hospital admissions. 	[394]
<ul style="list-style-type: none"> - COMBOS Trial: - subjects with Residual hypertriglyceridemia despite 8 weeks of diet and simvastatin 40mg/d therapy - (256) 	<ul style="list-style-type: none"> - P-OM3 4 g/d to an ongoing regimen of simvastatin 40 mg/d vs. simvastatin 40mg/day only - 16 weeks 	<ul style="list-style-type: none"> - P-OM3 significantly reduced VLDL-P size and increased low-density LDL-P size without altering HDL-P size. - P-OM3 did not significantly change total VLDL-P or LDL-P concentrations. - P-OM3 significantly lowered large VLDL-P and IDL-P concentrations. - P-OM3 significantly reduced Lp-PLA₂ concentrations. 	<ul style="list-style-type: none"> - High dose n-3 PUFA supplementation in conjunction with statin therapy may improve lipoprotein profiles and reduce inflammatory response in hypertriglyceridemic patients. 	[395]

<ul style="list-style-type: none"> - Hyperlipidemia - (34) 	<ul style="list-style-type: none"> - 7.5g DHA oil (3g DHA)/day vs. placebo - 3 months 	<ul style="list-style-type: none"> - Decreased circulating WBCs, CRP, GM-CSF and IL-6 concentrations. - Increased MMP-2 levels. - No significant change in plasma NO, SAA, G-CSF, IL-1b, IL-2, IL-8, IL-10, TNFα, ICAM-1, VCAM-1, and E-Selectin. 	<ul style="list-style-type: none"> - Short term DHA supplementation may alter the inflammatory response in dyslipidemic male patients by impacting circulating inflammatory biomarker levels which may correlate with improved blood lipid profile and fatty acid composition. 	[396]
<ul style="list-style-type: none"> - OCEAN Trial: - Patients awaiting carotid endarterectomy - (121) 	<ul style="list-style-type: none"> - OMACOR 2g/day (0.81g EPA and 0.675g DHA ethyl esters) vs. placebo - 21 days 	<ul style="list-style-type: none"> - Increased plaque EPA and decreased foam cell composition. - Negative correlation between plaque EPA composition and plaque inflammation, instability and number of plaque T cells. - Significantly lowered plaque MMP-7, MMP-9, MMP-12, IL-6, ICAM-1, and TIMP-2 mRNA levels. 	<ul style="list-style-type: none"> - Increased incorporation of EPA into atherosclerotic plaques in patients with advanced carotid atherosclerosis supplemented with n-3 PUFAs may be associated with reduced plaque inflammation and improved plaque stability. 	[225]
<ul style="list-style-type: none"> - DOIT Trial: - Cholesterol >6.45 mmol/L - (563) 	<ul style="list-style-type: none"> - 2.4g/day n-3 PUFAs (49% EPA and 35% DHA) vs. placebo - 3 years 	<ul style="list-style-type: none"> - Significantly reduced all-cause mortality (HR=0.53), as well as fatal and non-fatal CV events (HR=0.89). 	<ul style="list-style-type: none"> - Male patients with elevated serum cholesterol supplemented with n-3 PUFAs may experience reduced mortality and incidence of CV events. 	[397]
<ul style="list-style-type: none"> - MARINE Trial: - Elevated TG - (299) 	<ul style="list-style-type: none"> - AMR101 (EPA ethyl ester; icosapent-ethyl) 4g/day and 2g/day vs. placebo - 12 weeks 	<ul style="list-style-type: none"> - Significantly reduced TG levels from baseline <ol style="list-style-type: none"> 1. -33.1% (4g/day) 2. -19.7% (2g/day) 	<ul style="list-style-type: none"> - High and moderate dose EPA supplementation in patients with hypertriglyceridemia may reduce TG levels, improve overall blood lipid profile, and reduce PLA₂ activity levels. 	[398]

<ul style="list-style-type: none"> - Previous PCI - ACS or stable angina - (54) 	<ul style="list-style-type: none"> - Observational study. - No intervention 	<ul style="list-style-type: none"> - Higher colour grade of yellow plaques and number of non-culprit yellow plaques with thrombus in patients with low EPA:AA ratio. - Association between serum EPA and grade 3 yellow plaques (OR=0.98). 	<ul style="list-style-type: none"> - Low serum EPA and EPA/AA ratio may be correlated with the observed increase in coronary plaque grade and plaque vulnerability in patients with a history of CVD who have undergone PCI. 	[399]
<ul style="list-style-type: none"> - ANCHOR Trial: - High CVD risk - Statin therapy - Elevated TG - (702) 	<ul style="list-style-type: none"> - AMR101 (EPA ethyl ester; icosapent-ethyl) 4g/day and 2g/day vs. placebo - 12 weeks 	<ul style="list-style-type: none"> - Significantly reduced <ul style="list-style-type: none"> (A) TG levels <ol style="list-style-type: none"> 1. -21.5% (4g/day) 2. -10.1% (2g/day) (B) Lipoprotein Phospholipase A2 <ol style="list-style-type: none"> 1. -19.0% (4g/day) 2. -18.0% (2g/day) (C) hs-CRP <ol style="list-style-type: none"> 1. -22.0% (4g/day) 2. -6.8% (2g/day) 	<ul style="list-style-type: none"> - In high risk patients on statin therapy with elevated TG, EPA may improve plasma lipid parameters compared to baseline levels as well as reduce markers of systemic inflammation. 	[400]
<ul style="list-style-type: none"> - EVOLVE Trial: - Elevated TG - Untreated dyslipidemia or on stable dose lipid-lowering therapy - BMI \geq 20 - (399) 	<ul style="list-style-type: none"> - EPANOVA (n-3 free fatty acid) 2,3, and 4g/day vs. placebo - 12 weeks 	<ul style="list-style-type: none"> - Significantly reduced <ul style="list-style-type: none"> (A) TG levels <ol style="list-style-type: none"> 1. -25.9% (2g/day) 2. -25.5% (3g/day) 3. -30.9% (4g/day) (B) Lipoprotein Phospholipase A2 <ol style="list-style-type: none"> 1. -14.9% (2g/day) 2. -11.1% (3g/day) 3. -17.2% (4g/day) - No significant change in hs-CRP 	<ul style="list-style-type: none"> - N-3 PUFA supplementation in conjunction with lifestyle and diet interventions may improve serum lipid parameters but only have a modest effect on inflammatory response. 	[401]
<ul style="list-style-type: none"> - Untreated dyslipidemia with non-culprit thin-cap fibroatheroma (TCFA) lesions - Underwent PCI or ACS with elevated LDL-C - (30) 	<ul style="list-style-type: none"> - 1.8g/day EPA + rosuvastatin vs. rosuvastatin alone - 9 months 	<ul style="list-style-type: none"> - Greater fibrous cap thickness and decrease in lipid arc and lipid length. - Significantly reduced hs-CRP levels and PTX3 cytokine levels. - Lower incidence of macrophage accumulation. 	<ul style="list-style-type: none"> - EPA supplementation in addition to statin therapy may enhance fibrous cap stability possibly by reducing plaque inflammation and systemic inflammatory response compared to statin treatment alone. 	[402]

<ul style="list-style-type: none"> - OMEGA-REMODEL Trial: - Prior MI - (358) 	<ul style="list-style-type: none"> - LOVAZA 4g/day (1.86g EPA and 1.5g DHA ethyl esters) vs. placebo - 6 months 	<ul style="list-style-type: none"> - Significantly reduced <ol style="list-style-type: none"> 1. LV remodeling 2. hs-CRP 3. Lipoprotein Phospholipase A2 4. Myeloperoxidase levels 	<ul style="list-style-type: none"> - N-3 fatty acids may reduce the extent of myocardial remodeling and fibrosis as well as serum biomarkers of inflammation in patients post-MI. 	[403]
<ul style="list-style-type: none"> - Statin treatment for at least 6 months - Dyslipidemia, stable angina with plan to be treated with bare metal stent - (95) 	<ul style="list-style-type: none"> - 1.8g/day EPA + statin vs. statin alone - 6 months 	<ul style="list-style-type: none"> - Increased EPA:AA ratio from baseline. - Significant increase in fibrous volume and reduction in lipid volume of coronary plaques. - Significant decrease in PTX3 and MCP-1 levels. - Change in lipid volume significantly correlated with PTX3 cytokine and MCP-1 levels. 	<ul style="list-style-type: none"> - Treatment with EPA in conjunction with statin therapy may help improve coronary plaque stability and composition which may be associated with a reduction in local inflammatory biomarker concentrations. 	[204]
<ul style="list-style-type: none"> - REDUCE-IT Trial: - CVD or diabetes - Statin therapy - TG 1.52-5.56 mmol/L - LDL-C 1.06-2.59 mmol/L - (8179) 	<ul style="list-style-type: none"> - VASCEPA (EPA ethyl ester; icosapent-ethyl) 4g/day vs. placebo - 4.9 years 	<ul style="list-style-type: none"> - Significant reduction in CV death, non-fatal MI or stroke, coronary revascularization, or unstable angina (HR=0.75). - Significant reduction in hs-CRP. 	<ul style="list-style-type: none"> - High dose EPA may reduce incidence of major CV events or mortality in patients at high risk for or with established CVD. 	[404]

Table 2: Clinical trials showing no effect of n-3 PUFAs in the cascade of IHD

Sample criteria and size (n)	Treatment Protocol	Key Findings	Conclusion	Reference
<ul style="list-style-type: none"> - ALPHA OMEGA Trial: - Prior MI (median 3.7 years) - (4837) 	<ul style="list-style-type: none"> - EPA + DHA 0.4g/day vs. placebo - 3.4 years 	<ul style="list-style-type: none"> - No significant change in major CV events (HR=1.01). 	<ul style="list-style-type: none"> - Low dose dietary supplementation with n-3 PUFAs in patients with previous MI receiving optimized pharmacological therapy may be ineffective for secondary prevention of subsequent major cardiovascular events. 	[177]
<ul style="list-style-type: none"> - OMEGA Trial: - Recent MI (3-14 days) - Received guideline recommended treatment for acute MI - (3851) 	<ul style="list-style-type: none"> - 1g/day (0.46g EPA and 0.38g DHA) vs. placebo - 1 year 	<ul style="list-style-type: none"> - No significant change in sudden cardiac death (OR=0.95). 	<ul style="list-style-type: none"> - Patients receiving guideline-recommended post-MI pharmacological therapy and supplemented with n-3 PUFAs may not receive any additional benefit in prevention of sudden cardiac death, CV events, or total mortality compared to guideline pharmacological therapy alone. 	[178]
<ul style="list-style-type: none"> - Elevated TG - (26) 	<ul style="list-style-type: none"> - High dose n-3 PUFA 3.4g/day EPA+DHA (LOVAZA, 0.465 EPA and 0.375 DHA per 1g capsule) vs. low dose n-3 PUFA 0.85g/day EPA+DHA - 8 weeks 	<ul style="list-style-type: none"> - Decreased TG (-27%). - No significant changes in measures of endothelial function, cholesterol, hs-CRP, IL-1β, IL-6, and TNFα levels or gene expression in lymphocytes. 	<ul style="list-style-type: none"> - Supplementation with high dose n-3 PUFAs may lower TG levels from baseline but have little additional benefit on endothelial function or inflammation compared to low dose n-3 PUFA supplementation. 	[405]
<ul style="list-style-type: none"> - ORIGIN Trial: - High CVD risk - Dysglycemia or diabetes - (12,536) 	<ul style="list-style-type: none"> - 0.9g/day EPA and DHA vs. placebo - 6.2 years 	<ul style="list-style-type: none"> - No significant reduction in CV death (HR=0.98). 	<ul style="list-style-type: none"> - N-3 PUFA supplementation in patients with dysglycemia at high risk for CVD may not significantly reduce the incidence of major CV events or mortality despite a reduction in TG levels. 	[181]

<ul style="list-style-type: none"> - SU.FOL.OM3 Trial: - Prior MI or ischemic stroke (1-12 months) - (2501) 	<ul style="list-style-type: none"> - 0.6g/day EPA and DHA vs. placebo - 4.2 years 	<ul style="list-style-type: none"> - No significant reduction in CV death, non-fatal MI, or stroke (HR=1.08). 	<ul style="list-style-type: none"> - Secondary prevention of coronary-related events may not be significantly reduced by n-3 PUFAs. 	[406]
<ul style="list-style-type: none"> - R&P Trial: - CV risk factors, - clinical atherosclerosis, - no previous MI - (12,513) 	<ul style="list-style-type: none"> - 1g/day EPA and DHA vs. placebo - 5 years 	<ul style="list-style-type: none"> - No significant reduction in CV death or hospital admission (HR=0.97). 	<ul style="list-style-type: none"> - N-3 PUFAs may not be beneficial for the primary prevention of CV events or death in patients with multiple CV risk factors. 	[407]
<ul style="list-style-type: none"> - ASCEND Trial: - Diabetes - No CVD or atherosclerosis - (15,480) 	<ul style="list-style-type: none"> - 1g/day n-3 PUFAs (460mg EPA and 380mg DHA) vs. placebo - 7.4 years 	<ul style="list-style-type: none"> - No significant difference between first serious CV event rates (RR=0.97). 	<ul style="list-style-type: none"> - N-3 PUFA supplementation may be ineffective as a primary prevention strategy of CV events in patients with type 2 diabetes mellitus. 	[408]
<ul style="list-style-type: none"> - VITAL Trial: - No CV event or cancer history - (25,871) 	<ul style="list-style-type: none"> - OMACOR 1g/day (0.46g EPA and 0.38g DHA) vs. placebo - 5.3 years 	<ul style="list-style-type: none"> - No significant difference in major CV events (HR=0.92). 	<ul style="list-style-type: none"> - N-3 supplementation may not provide any benefit in the primary prevention of major CV events regardless of a patient's CVD risk. 	[409]

Abbreviations (Table 1 and 2):

ACS, Acute coronary syndrome; BMI, Body mass index; CAD, Coronary artery disease; CETP, Plasma cholesterol ester transfer protein; CRP, C-reactive protein; CV, Cardiovascular; CVD, Cardiovascular disease; G-CSF, Granulocyte-colony stimulating factor; GM-CSF, Granulocyte monocyte-colony stimulating factor; HR, Hazard ratio; ICAM-1, Intercellular adhesion molecule-1; IDL-P, Intermediate density lipoprotein; IHD, Ischemia heart disease; IL, Interleukin; LDL-C, Low density lipoprotein cholesterol; Lp-PLA₂, Lipoprotein phospholipase A₂; LV, Left ventricular; MCP-1, Monocyte chemoattractant protein-1; MI, Myocardial infarction; MMP, Matrix metalloproteinase; NO, Nitric oxide; NYHA, New York Heart Association; OA, Osteoarthritis; OR, Odds ratio; P-OM3, Prescription omega-3-acid ethyl esters; PCI, Percutaneous coronary intervention; PL, Phospholipid; PTX3, Pentraxin-3; PUFA, Polyunsaturated fatty acid; RA, Rheumatoid arthritis; RLP-TG, Remnant lipoprotein triglycerides; RR, Relative risk; SAA, Serum amyloid A; sdLDL, Small dense low density lipoprotein; T2DM, Type 2 diabetes mellitus; TC, Total cholesterol; TG, Triglycerides; TIMP, Tissue inhibitor of MMP; TNF α , Tumor necrosis factor α ; VCAM-1, Vascular cell adhesion molecule-1; VLDL-P, Very low density lipoprotein.

Table 3: Examples for the immunomodulatory effects mediated by n-3 PUFA and its metabolites

Atherosclerosis and Thrombosis				
N-3 PUFA / Metabolite	Experimental Model	Effects on Different Immune Components	Conclusion	Reference
EPA or DHA	<ul style="list-style-type: none"> - Human platelet-rich plasma - EPA (IC₅₀ = 5.9 µM) or DHA (IC₅₀ = 2.2 µM) added for 90 seconds - Aggregation stimulated using stable TxA₂ mimetic, U46619, fibrinogen and CaCl₂. 	<ul style="list-style-type: none"> - DHA and EPA antagonized TxA₂ and PGH₂ receptor which decreased platelet aggregation. - DHA was found to be more potent than EPA in blocking platelet aggregation. 	- EPA and DHA are capable of directly inhibiting agonist interaction with Tx receptor and thus inhibit platelet activation which may serve as a mechanism for their anti-thrombotic effects.	[410]
DHA	<ul style="list-style-type: none"> - HSAVECs or HUVECs - Pre-treatment with DHA (10 µM) for 24 to 96h - Challenging with the cytokines IL-1α, IL-1β, IL-10, TNF-α, IL-4 or bacterial LPS for an additional 0 to 24h. 	<ul style="list-style-type: none"> - DHA inhibited endothelial activation. - DHA decreased cytokine-induced expression of endothelial and leukocyte adhesion molecules VCAM-1, E-selectin and ICAM-1. - DHA decreased secretion of the inflammatory mediators IL-6 and IL-8. - DHA reduced the adhesion of human monocytes to cytokine-stimulated endothelial cells. 	- The anti-inflammatory effects of DHA may contribute to its ability to inhibit the development as well as the progression of atherosclerosis.	[212]
EPA and DHA	<ul style="list-style-type: none"> - Monocytes obtained from healthy non-smoking adults - Pretreated with a mixture of EPA and DHA at a final ratio 3 EPA : 2 DHA (39µM EPA: 26µM DHA) - Challenged with IFN-γ for 48h. 	<ul style="list-style-type: none"> - Mixture inhibited MHC II and ICAM-1. - Mixture reduced CD4 cell activation. 	- N-3 PUFAs are efficient in protection against monocyte activation.	[411]
EPA	<ul style="list-style-type: none"> - Human monocytic THP-1 cells - Pre-incubated with EPA (60 µM) - Stimulated with LPS for various time periods (6, 12 and 24h) 	- EPA attenuated TNF-α production by inhibiting NF-κB activation and binding to DNA.	- EPA might be used for the prevention and alleviation of atherosclerosis as well as the associated-thrombotic episodes.	[218]

Menhaden fish oil (n-3 PUFA: 16% EPA, 19% EPA)	<ul style="list-style-type: none"> - WT and PPARα-null mice (A) Chronic treatment: Unrestricted access to menhaden fish oil diet for 15 days (B) Acute treatment: Two oral doses of fish oil (0.01 ml/g) at 0 and 12h in a 24h study. 	<ul style="list-style-type: none"> - Fish oil stimulated the secretion of the anti-inflammatory and the anti-atherogenic hormone adiponectin by two folds. - This effect is PPARγ-dependent and PPARα-independent. 	N-3 PUFAs possess anti-inflammatory and antiatherogenic properties which could be mediated by stimulation of adiponectin secretion.	[412]
EPA	<ul style="list-style-type: none"> (A) In vivo experiment: <ul style="list-style-type: none"> - ApoE$^{-/-}$ or LDL-R$^{-/-}$ mice - Fed on western-type diet \pm 5% EPA (w/w) (5.35 kcal/g) for 13 weeks (B) In vitro experiment: <ul style="list-style-type: none"> - HUVEC, human monocytic THP-1 cells, and murine macrophage RAW264.7 cells - Pre-treated with EPA (1 μM) for 48h - Stimulated with TNF-α (10 ng/ml) for 24h by LPS (10 ng/mL) for 4h. 	<ul style="list-style-type: none"> - EPA suppressed the development of atherosclerotic lesions. - EPA stabilized atherosclerotic plaque. - EPA decreased macrophage infiltration in conjunction with increased collagen. - EPA abolished MMP production in macrophage like cells. - EPA upregulated PPARα and thus inhibited NF-κB activation. - EPA attenuated the up-regulation of VCAM-1, ICAM-1 and MCP-1 in HUVECs as well as the expression of MMP-2 and MMP-9 in macrophage-like cells induced by TNF-α. 	- The anti-inflammatory effects of EPA accounts for its ability to prevent the progression and the destabilization of atherosclerotic lesions.	[201]
ALA (7.3% w/w)	<ul style="list-style-type: none"> - WT mice - Fed a 0.21% (w/w) cholesterol diet containing either a high (7.3%) or low (0.03%) ALA concentration for 2 weeks - Subjected to photochemical injury for the induction of thrombosis. 	<ul style="list-style-type: none"> - ALA decreased impaired arterial thrombus formation, platelet activation, and NF-κB activity in mice. 	- ALA represents an attractive nutritional intervention with direct antithrombotic effects in cardiovascular disorders.	[413]

EPA	<ul style="list-style-type: none"> - HUVEC - Treated with EPA (3, 10 or 30 μM) in addition to PA (100 μM) for 1 day 	<ul style="list-style-type: none"> - EPA suppressed the PA-induced upregulation of ICAM-1, MCP-1, and IL-6. - EPA inhibited NF-κB pro-inflammatory pathway. 	<ul style="list-style-type: none"> - EPA attenuates saturated fatty acid-induced macrophage cytokine production as well as vascular endothelial dysfunction which explain the anti-atherogenic action of EPA in the clinical setting. 	[414]
RvE1	<ul style="list-style-type: none"> - Female ApoE3 Leiden transgenic mice - Fed an atherogenic diet for 9 weeks - Treated with either low (1mg/kg/day) or high (5mg/kg/day) RvE1 supplements. 	<ul style="list-style-type: none"> - RvE1 reduced atherosclerotic lesions. - RvE1 did not affect plasma E-selectin, VCAM-1 or MCP-1 levels. - RvE1 reduced plasma EPHX4 levels. - RvE1 down-regulated the local expression of pro-atherogenic genes in the aorta. - RvE1 inactivated IFN-γ and TNF-α signalling pathways in the aorta. 	<ul style="list-style-type: none"> - RvE1 has local anti-inflammatory effects within the aorta and thus attenuates atherogenesis without affecting plasma lipids. 	[378]
<ul style="list-style-type: none"> - EPA alone - DHA alone - EPA+DHA 	<p>(A) In vivo:</p> <ul style="list-style-type: none"> - ApoE -/- mice - Fed western diet supplemented with either <ol style="list-style-type: none"> 1. EPA (2.5%, w/w), 2. Low-dose EPA + DHA (2.5%, w/w), or 3. High-dose EPA + DHA (5%, w/w) for 20 weeks. <p>(B) In Vitro:</p> <ul style="list-style-type: none"> - RAW264.7 cells - Pretreated with EPA (3 μM) or DHA (3 μM) or their combination for 48h - Challenged by LPS (10 ng/mL) for 4h. 	<ul style="list-style-type: none"> - EPA and DHA additively: <ol style="list-style-type: none"> 1. Stabilized atherosclerotic plaque 2. Decreased lipid accumulation 3. Attenuated the expression of inflammatory molecules 4. Inhibited macrophage activation 5. Suppressed LPS-induced TLR4 expression in lipid rafts on RAW264.7 cells - Decreased LPS-induced elevation of MCP1, TNFα and MMP9. 	<ul style="list-style-type: none"> - DHA has additional anti-atherosclerotic effects when combined with EPA. - DHA attenuates pro-inflammatory activation of macrophages by inhibiting TLR4 accumulation into lipid rafts. 	[242]
Myocardial Infarction				
RvD1	<ul style="list-style-type: none"> - WT mice 	<ul style="list-style-type: none"> - RvD1 reduced neutrophil density in the 	<ul style="list-style-type: none"> - RvD1 exerts potent pro- 	[388]

	<ul style="list-style-type: none"> - Subjected to the surgical ligation of LAD to induce MI - Injected with either Lipo-RvD1 (3 µg/kg/day) or free RvD1 (3 µg/kg/day) 3h post-MI and monitored for 5 days. 	<ul style="list-style-type: none"> spleen and discontinued neutrophil recruitment in LV post-MI. - RvD1 stimulated macrophage clearance from infarcted area and promoted early resolution of inflammation post-MI. - RvD1 reduced collagen deposition. - RvD1 liposomal formulation offered stability for long-term inflammation resolving effect. 	<ul style="list-style-type: none"> resolving actions in MI-mediated injury and thereby limited the progression towards LV dysfunction post-MI. 	
Ischemia-Reperfusion Injury				
RvE1	<p>(A) In vivo:</p> <ul style="list-style-type: none"> - Sprague-Dawley rats - Underwent 30 min of ischemia (LAD ligation) and 4h of reperfusion. - Before reperfusion, rats received intravenous RvE1 (0, 0.03, 0.1, or 0.3mg/kg). <p>(B) In vitro:</p> <ul style="list-style-type: none"> - H9c2 cells - Incubated with RvE1 (0, 1, 10, 100, or 1000 nM) - Subjected to 18h normoxia, 16h of hypoxia, or 16h of hypoxia and 2h of reoxygenation. 	<ul style="list-style-type: none"> - RvE1 reduced infarct size. - RvE1 reduced leukocyte infiltration to the ischemic site. 	<ul style="list-style-type: none"> - RvE1 has a direct protective effect on cardiomyocytes against IR injury. 	[384]
EPA	<ul style="list-style-type: none"> - Pigs - Treated with EPA chow (600mg/kg/day) for 3 weeks - Subjected to myocardial ischemia by 90-min occlusion of the left circumflex coronary artery and subsequent 60-min reperfusion. 	<ul style="list-style-type: none"> - EPA ameliorated myocardial IR injury. - EPA inhibited myocardial Rho-kinase activity. 	<ul style="list-style-type: none"> - Long-term treatment with EPA ameliorates IR injury partly through Rho-kinase pathway inhibition. 	[415]
RvD1	<ul style="list-style-type: none"> - Sprague-Dawley rats. - Ischemia: LAD occlusion for 40 min. 	<ul style="list-style-type: none"> - RvD1 decreased neutrophil infiltration into infarcted region. - RvD1 decreased the release of pro- 	<ul style="list-style-type: none"> - Single RvD1 dose, given 5 min before occlusion or 5 min after the onset of reperfusion, 	[387]

	<ul style="list-style-type: none"> - Reperfusion for 19 days. - 0.1 µg RvD1 injected into the LV cavity 5 min before ischemia or 5 min after the onset of reperfusion. 	inflammatory cytokines.	decreases the infarct size post-MI.	
19,20-EDP	<ul style="list-style-type: none"> - Isolated mouse hearts perfused in the Langendorff mode - Perfused with 1 µM 19,20-EDP - Subjected to 30 min ischemia and followed by 40 min of reperfusion. 	- 19,20-EDP inhibited the activation and assembly of NLRP3 inflammasome with its downstream detrimental signals, caspase 1 and IL-1β under IR conditions.	- 19,20-EDP provides protection against IR injury via inhibiting the detrimental NLRP3 inflammasome responses.	[82]
Cardiac Fibrosis and Remodeling				
EPA	<ul style="list-style-type: none"> - Neonatal rat ventricular cardiomyocytes - Pretreated with 10 µM EPA, - Challenged with 0.1 nM ET-1, on day 5 of culture for 24h 	<ul style="list-style-type: none"> - EPA attenuated TGF-β1 and JNK upregulation caused by ET-1. - EPA reduced ET-1 induced hypertrophy. 	- Fish oil may have beneficial protective effects on cardiac hypertrophy.	[416]
EPA + DHA	<ul style="list-style-type: none"> - WT mice - Fed a fish oil-supplemented diet (12 g menhaden oil + 28 g corn oil per kg) for 8 weeks - Subjected to TAC surgery - Mice continually fed the fish oil-supplemented diet and euthanized after 3, 7, or 28 days. 	- Fish oil inhibited TGF-β1 induced transformation and proliferation of cardiac fibroblasts and thus reduced cardiac fibrosis.	- N-3 PUFAs prevent cardiac fibrosis and cardiac dysfunction by blocking TGF-β1 -induced phospho-Smad2/3 nuclear translocation.	[417]
EPA or DHA	<ul style="list-style-type: none"> - WT mice - Started on diets supplemented with 1.9 g/Kg EPA or 1.3 g/Kg DHA for 2 weeks - Subjected to TAC surgery - Diets continued for an additional 6 weeks. 	- EPA, not DHA, inhibited TGF-β1 signalling via activation of GRP120 and thus ameliorated cardiac fibrosis.	- EPA-mediated prevention of fibrosis via activation of GRP120 could represent a novel therapy for HFpEF.	[227]
EPA	<ul style="list-style-type: none"> - DS/obese rat - Fed with low dose (300 mg/kg) or high dose (1g/kg) of EPA for 4 weeks 	<ul style="list-style-type: none"> - EPA increased adiponectin secretion which inactivated NF-kB. - EPA reduced cardiac fibrosis as well as ameliorated LV fibrosis, diastolic dysfunction, oxidative stress and inflammation without lowering blood 	- EPA may be suitable for the treatment of cardiac injury associated with metabolic syndrome.	[229]

		pressure in DS/obese rats.		
EPA	<ul style="list-style-type: none"> - WT mice. - Oral doses of EPA (1g/kg) for 28 days. - Subjected to MI by LAD ligation. - Mice treated again with EPA (1g/kg) once daily for 28 days after MI. 	<ul style="list-style-type: none"> - EPA attenuated fibrosis in the myocardium. - EPA decreased expression of fibrotic genes. - EPA inhibited TGFβ/Smad signalling. 	<ul style="list-style-type: none"> - Long-term administration of EPA improves the prognosis and attenuates chronic cardiac remodeling after MI by modulating the activation of proinflammatory M1 macrophages. 	[228]
DHA or RvD1	<ul style="list-style-type: none"> - Cultured cardiac fibroblasts and peritoneal macrophages isolated from WT and 12/15LOX$^{-/-}$ mice - Treated for 4, 8, 12, and 24h with: <ol style="list-style-type: none"> 1. DHA (50 μM), or 2. RvD1 (10 ng/ml) 	<ul style="list-style-type: none"> - RvD1 showed higher potential than DHA in limiting pro-inflammatory cytokines and chemokine in the absence of 12/15LOX. - RvD1 polarized macrophages towards resolving phenotype (M2) than DHA. - RvD1, but not DHA, diminished expression of COX-2 in 12/15LOX$^{-/-}$ cardiac fibroblast. 	<ul style="list-style-type: none"> - RvD1 possess potent immunomodulating properties, even stronger than DHA, and is a potential candidate for therapeutics in inflammatory diseases including cardiac remodeling. 	[418]

Abbreviations:

MHC, Major histocompatibility complex; MMP, Matrix metalloproteinase; NRVC, Neonatal rat ventricular cardiomyocytes; PA, Palmitic acid; PGH₂, Prostaglandin H₂; PPAR, Peroxisome proliferator-activated receptor; PUFA, Polyunsaturated fatty acids; Rv, Resolvin; Smad, Suppressor of mothers against decapentaplegic; TAC, Transverse aortic constriction; TLR, Toll-like receptor; TNF α , Tumor necrosis factor α ; TGF- β 1, Transforming growth factor- β 1; TxA₂, Thromboxane A₂; VCAM-1, Vascular cell adhesion molecule-1

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Figure 1

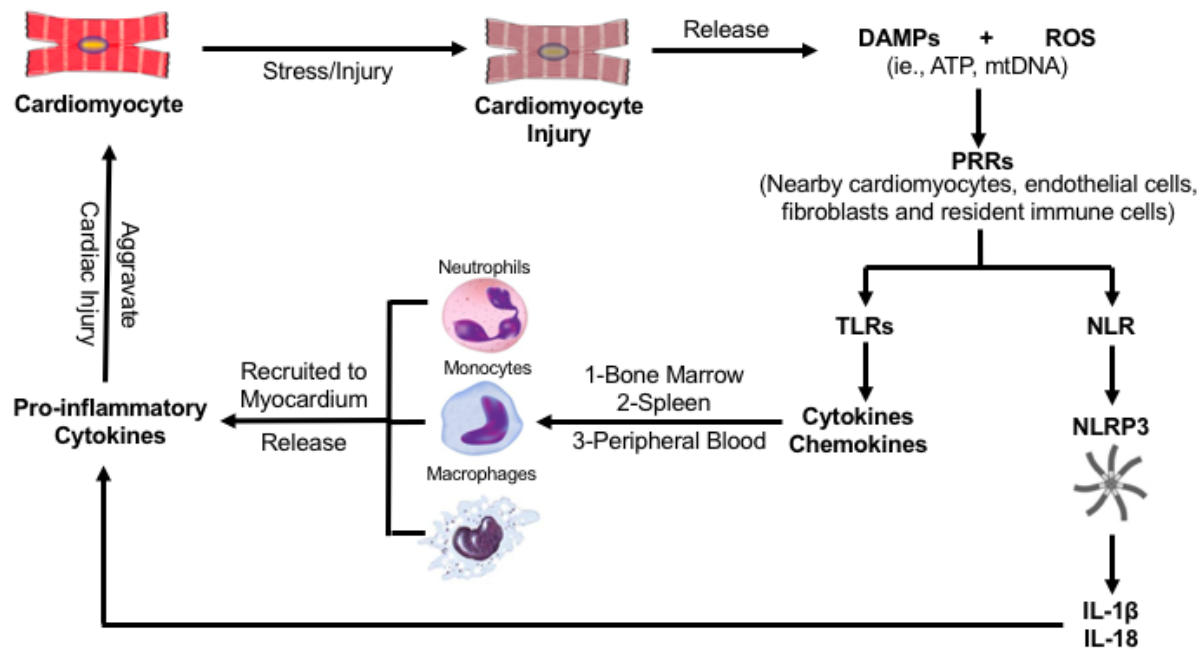


Figure 2

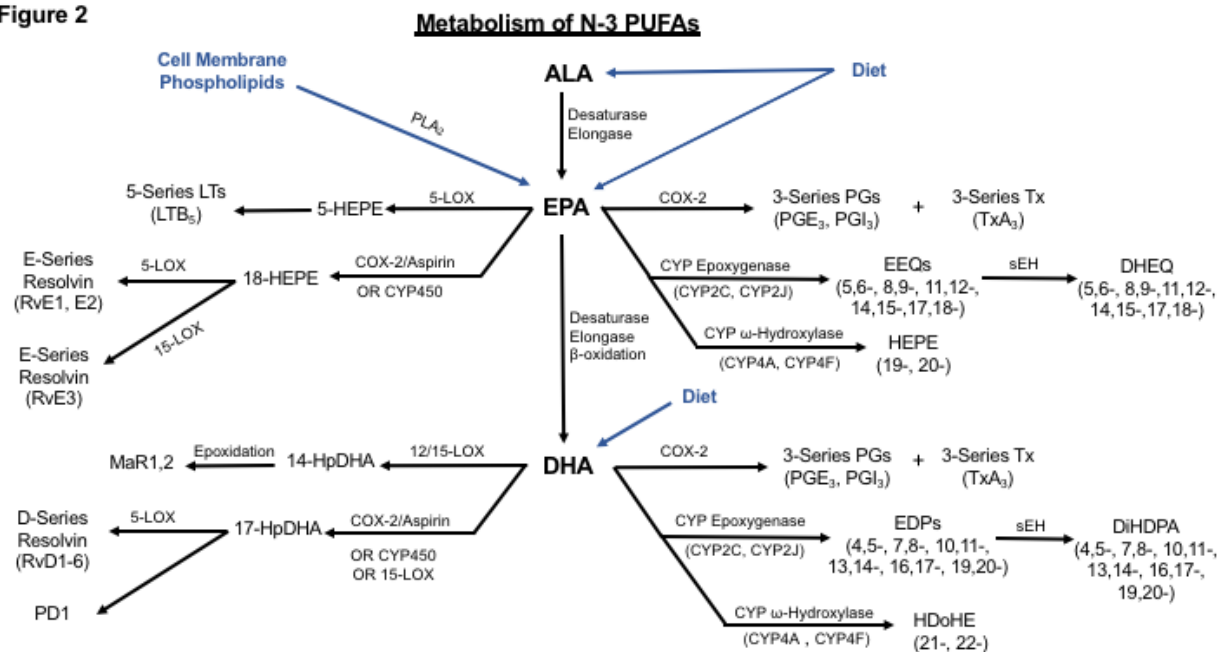


Figure 3

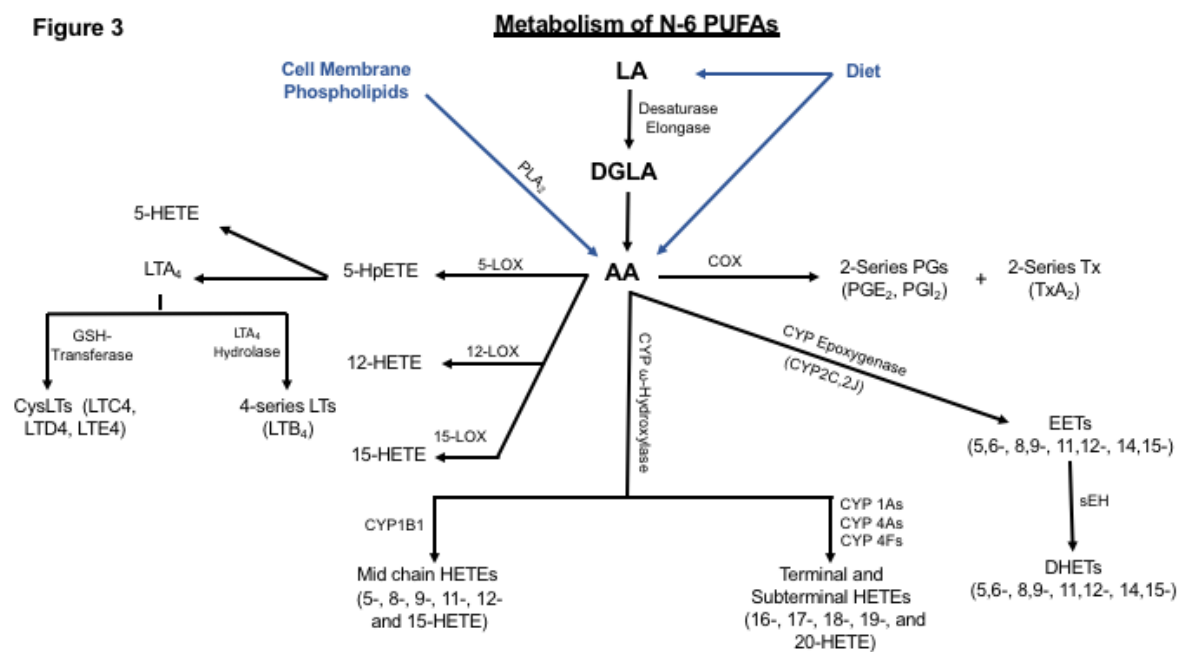
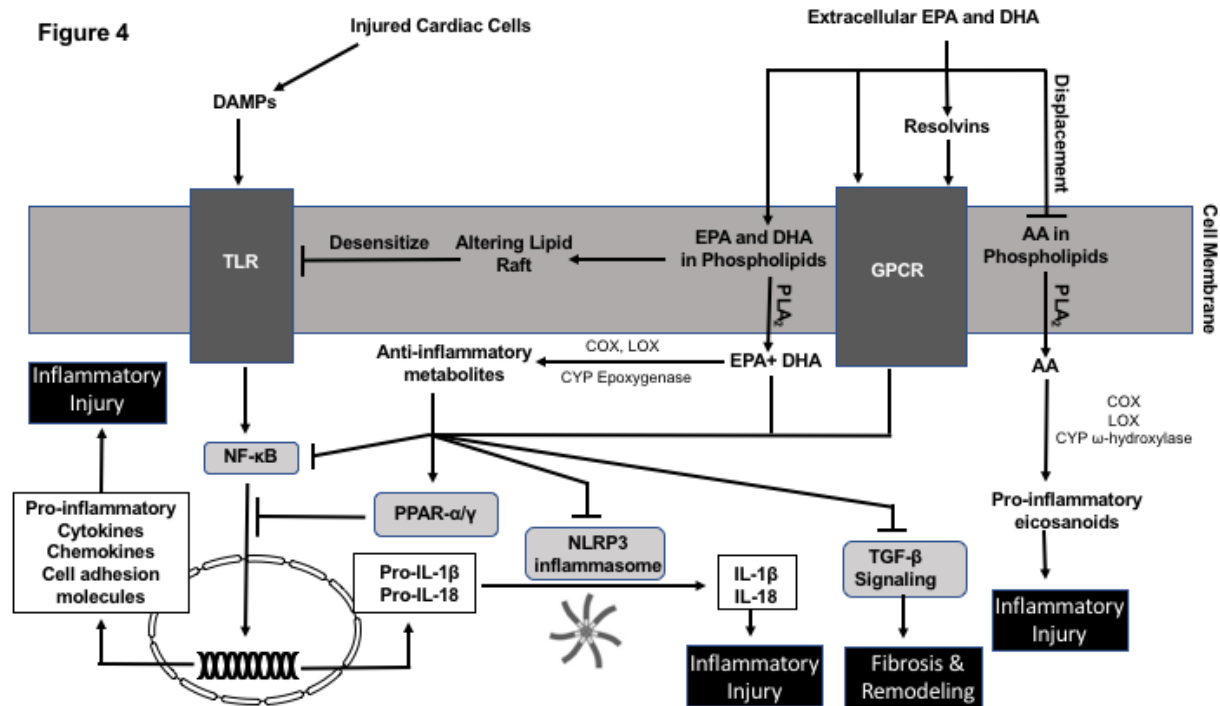


Figure 4



Highlights

- Excessive activation of the innate immune system may worsen adverse effects from ischemic injury
- Modulating the innate immune system can limit adverse effects from ischemic injury
- N-3 PUFAs are metabolized to bioactive lipid mediators that possess anti-inflammatory properties
- Cardioprotective effects of n-3 PUFAs against ischemic injury involve immunomodulatory properties

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Nothing to declare.