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Novel Approaches for Omega-3 Fatty Acid Therapeutics: Chronic Versus Acute Administration to Protect Heart, Brain, and Spinal Cord

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Keywords

omega-3 fatty acids, stroke, myocardial infarction, spinal cord and brain injury, acute treatment, chronic treatment

Abstract

This article reviews novel approaches for omega-3 fatty acid (FA) therapeutics and the linked molecular mechanisms in cardiovascular and central nervous system (CNS) diseases. In vitro and in vivo research studies indicate that omega-3 FAs affect synergic mechanisms that include modulation of cell membrane fluidity, regulation of intracellular signaling pathways, and production of bioactive mediators. We compare how chronic and acute treatments with omega-3 FAs differentially trigger pathways of protection in heart, brain, and spinal cord injuries. We also summarize recent omega-3 FA randomized clinical trials and meta-analyses and discuss possible reasons for controversial results, with suggestions on improving the study design

for future clinical trials. Acute treatment with omega-3 FAs offers a novel approach for preserving cardiac and neurological functions, and the combinations of acute treatment with chronic administration of omega-3 FAs might represent an additional therapeutic strategy for ameliorating adverse cardiovascular and CNS outcomes.

Contents

1. INTRODUCTION: OMEGA-3 FATTY ACIDS

CVD: cardiovascular disease

SCI: spinal cord injury

TBI: traumatic brain injury

FA: fatty acid

RCT: randomized clinical trial

EPA: eicosapentaenoic acid

DHA: docosahexaenoic acid

Cardiovascular diseases (CVDs) are a leading cause of mortality worldwide, accounting for more than 17.6 million deaths in 2016, with a sharp increase of 14.5% from 2006. Among all-cause CVD mortality, 5.5 million deaths were attributed to cerebrovascular disease worldwide (2.7 million deaths from ischemic stroke and 2.8 million deaths from hemorrhagic stroke) (14). Within the field of neurotrauma, spinal cord injury (SCI) and traumatic brain injury (TBI) also lead to major disability and mortality, with significant socioeconomic costs and affecting younger age groups, comparable to that of CVD (51, 129). Data reported by the National Spinal Cord Injury Statistical Center in 2016 indicate that there are approximately 17,000 new SCI cases each year in the United States (51), and data on TBI show approximately 2.8 million events occurred in the United States in 2013 (129).

The effects of omega-3 fatty acids (FAs) on modulating the risks of CVD have been studied extensively through in vitro and animal experiments, controlled feeding studies, epidemiologic studies, and randomized controlled trials (RCTs) in humans. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), major representatives of this lipid class, are concentrated in oils

from marine fish, krill, and microalgae and are generally far more bioactive than their precursor, alpha linolenic acid (109).

Among their multiple CVD-related beneficial physiological effects, omega-3 FAs lower plasma triglycerides by reducing hepatic very low-density lipoprotein synthesis and by decreasing triglyceride synthesis as well as de novo lipogenesis; they also increase β-oxidation and reduce delivery of nonesterified FAs to the liver (64). Therefore, for patients with elevated triglyceride levels, the American Heart Association recommends intakes of >3 g of EPA and DHA per day as a monotherapy or as adjuncts to other lipid-lowering agents (121).

CNS: central nervous system

FO: fish oil

Experimental in vitro and in vivo studies suggest that lower heart rate and blood pressure mediated by omega-3 FAs results from direct effects on cardiac excitability through modulating the activity of multiple ion channels and stabilizing the cardiomyocyte membranes, indirect effects through improving left ventricular diastolic filling and augmenting vagal tone, or both (22, 52). Furthermore, these FAs improve endothelial function by stimulating translocation and activation of endothelial nitric oxide synthase into the cytosol and causing subsequent vasodilation (9).

Omega-3 FAs exhibit anti-inflammatory and antithrombotic properties; they incorporate into membrane phospholipids with a concomitant reduction in arachidonic acid (AA), the major bioactive representative of the omega-6 FA class, and compete with cyclooxygenase (COX) pathways. This step leads to a decreased production of omega-6 AA-derived inflammatory mediators. Also, omega-3 FAs are precursors of resolvins, protectins, and other bioactive mediators, which have potent anti-inflammatory properties and assist in the resolution of inflammation in cardiovascular and central nervous system (CNS) diseases (11, 115, 116). Furthermore, omega-3 FAs, as antithrombotic molecules, reduce platelet activation and adhesion, thromboxane A2 (TXA2) synthesis, and plasminogen activator inhibitor-1 plasma concentrations (72, 104).

As neuroprotectants, omega-3 FAs target several interrelated molecular mechanisms involving anti-inflammatory activity; oxidative stress reduction; and preservation of neurogenesis, synaptogenesis, and white matter integrity (123, 136). Of interest, EPA lowers the risk of cardioembolic stroke by affecting clotting processes and decreasing atrial fibrillation, and DHA plays a key role in reducing atherothrombotic stroke risk by reducing endothelial dysfunction (93, 112). Thus, it is important to consider DHA and EPA as biologically distinct molecules, and specific mechanisms underlying the differential effects mediated by DHA or EPA on stroke subtypes are of high interest and require further investigation.

There is substantial evidence that these FAs also have therapeutic potential in neurotrauma, as recent studies have shown that their acute administration by intravenous (IV) route after injury or dietary exposure before or after injury improves neurological outcomes in experimental animal models of SCI and TBI (88). The underlying mechanisms are associated with decreased neuroinflammation and oxidative stress, neurotrophic support, and activation of cell survival signaling pathways (88).

This review focuses on the effects of omega-3 FAs on the prevention of cardiovascular and CNS diseases and their potential to treat acute injuries related to heart, brain, and spinal cord. Molecular mechanisms involved in the chronic versus acute administration of these FAs are reviewed and compared. We also address potential strategies for successful clinical translation.

2. CHRONIC ADMINISTRATION OF OMEGA-3 FATTY ACIDS

Several primary and secondary prevention clinical trials as well as animal studies have indicated that consumption of fatty fish, fish oils (FOs), or individual omega-3 FAs could represent an effective dietary intervention to lower CVD morbidity and mortality. However, omega-3 FA concentrations in plasma and their content in cells and tissues are slowly responsive to omega-3 FA

consumption over time (59, 74). Supplementation with DHA ethyl esters or DHA triglycerides required 1 to 3 months to substantially increase DHA concentrations in plasma and red blood cells in humans (138). Incorporation of EPA and DHA into the membrane phospholipids in immune cells increased with FO consumption over 30 days in healthy human subjects (67). Similarly, maximum incorporation of omega-3 FAs in cardiac phospholipids could be achieved in rats after 8 weeks of oral ingestion (6). Thus, oral intake or ingestion of omega-3 FA supplements requires a prolonged period, over days to weeks, to achieve substantial cellular enrichment through which they may yield protective effects on CVD. Several molecular mechanisms accounting for the cardio- and neuroprotective effects of chronic omega-3 FA consumption have been proposed and are described below and depicted in **Figure 1**.

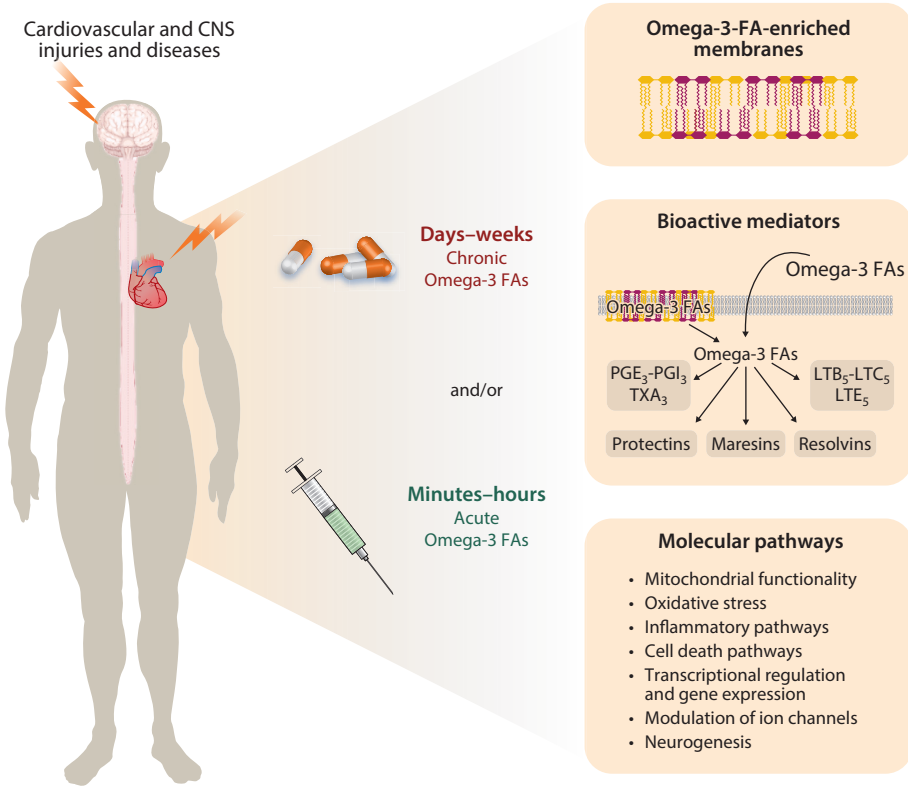

Figure 1

Molecular pathways modulated by chronic and acute administration of omega-3 FAs. Both chronic and acute administration of omega-3 FAs show protective effects on the prevention and treatment of cardiovascular and CNS injuries and diseases. However, whereas enrichment of omega-3 FAs into cell membranes through diet or supplements (chronic administration) occurs over days to weeks (59, 74, 138), infusing these FAs intravenously (acute administration) shows rapid increases in cell and tissue omega-3 FA contents within minutes to hours (26, 105). The potential underlying molecular pathways in common between chronic and acute administration of omega-3 FAs are associated with enrichment of cell membranes with omega-3 FAs and synthesis of lipid bioactive mediators, as well as preservation of mitochondrial integrity, reduction of oxidative stress, decrease of proinflammatory response, promotion of cell survival pathways, transcriptional regulation, modulation of ion homeostasis, and neurogenesis. Abbreviations: CNS, central nervous system; FA, fatty acid; LTB_5, leukotriene B_5; LTC_5, leukotriene C_5; LTE_5, leukotriene E_5; PGE_3, prostaglandin E_3; PGI_3, prostaglandin I_3; TXA_3, thromboxane A_3.

2.1. Inflammatory Pathways

Omega-3 FAs affect inflammatory processes either directly via modulation of transcription factors and gene expression or indirectly through their bioactive derivatives, such as eicosanoids, docosanoids, and other inflammation-resolving lipid mediators. Importantly, omega-3 FAs downregulate the activity of nuclear factor kappa B (NF-κB), a master transcriptional regulator of inflammatory responses involved in the pathogenesis of CVD (23, 38). Once translocated into the nucleus, NF-κB activates the gene expression of numerous proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) (38). Supplementation with EPA and DHA reduced circulating levels of TNF-α, IL-1, and IL-6 in aging patients with chronic systemic inflammation (127). Further, omega-3-activated peroxisome proliferator-activated receptor alpha and gamma (PPARα, PPARγ) reduce the expression of several inflammatory genes by inhibiting NF-κB activation (95, 148). It has been suggested that NF-κB activity is modulated by adiponectin, a cardioprotective adipocyte-derived hormone, and circulating adiponectin levels are elevated in a dose-dependent fashion with supplementation with EPA and DHA in animals (44) and humans (61). Omega-3 FAs may also regulate cardio- and neuroprotective signaling pathways through the activation of G-protein-coupled receptors (GPRs) (90). Available literature indicates that DHA interacts with GPR120, subsequently modulating NF-κB-mediated inflammatory pathways. Ren et al. (111) showed that a 2-week diet with DHA prior to ischemic brain injury in mice activated GPR120 and inhibited the inflammatory response in microglia, whereas knockdown of GPR120 exacerbated the inflammation induced by ischemic insult and abolished the anti-inflammatory effects of DHA.

NF-κB: nuclear factor kappa B

TNF: tumor necrosis factor

IL: interleukin

MI: myocardial infarction

SPM: specialized proresolving mediator

Eicosanoids and docosanoids produced from EPA and DHA are anti-inflammatory, compared with their AA-derived counterparts, and serve as vasodilators and inhibitors of platelet aggregation (119, 142). In contrast, omega-6-FA-derived mediators are generally proinflammatory and elicit adverse responses such as vasoconstriction, vasodilation, activation of leukocytes, stimulation of platelet aggregation, and generation of reactive oxygen species (ROS). Omega-6 AA is a substrate for COX enzymes to produce prostaglandins and TXA2, and 5-lipoxygenase (5-LOX) catalyzes the oxygenation reaction of AA to four-series leukotrienes and hydroxyleicosatetraenoic acids (7). Also, conversion of AA in the presence of 12-LOX increases the production of inflammatory cytokines, such as TNF-α, IL-1, or IL-6. Omega-3 FAs, in contrast, can reduce the production of AA-derived eicosanoids by competing with AA for incorporation into cell membrane phospholipids or by inhibiting COX-2 and 5-LOX enzymes. Administration of EPA 4 weeks before and 4 weeks after experimentally induced myocardial infarction (MI) attenuated later cardiac remodeling by reducing the activation of proinflammatory macrophages (126). In a mouse model of stroke, oral administration of DHA over 3 months helped prevent microglial activation and induced significant decreases in the levels of COX-2 and IL-1β after injury (73). Likewise, mice given long-term dietary supplementation with omega-3 FAs for 2 months prior to induction of TBI elicited robust neuroprotection, compared with the control group, and this was associated with a reduction in inflammatory markers, such as IL1-α, IL1-β, and TNF-α (106).

Over the past decade, specialized proresolving mediators (SPMs) have been increasingly recognized as a potent group of autacoids produced endogenously from the enzymatic oxygenation of EPA and DHA (116). SPMs, classified as protectins, resolvins, and maresins, elicit anti-inflammatory effects by inhibiting neutrophil transmigration and infiltration and by enhancing macrophage uptake of apoptotic cells and inhibiting inflammatory mediator synthesis. The major mechanisms exerted by these bioactive mediators are summarized in **Figure 2**. To determine whether supplementation with omega-3 FAs affects levels of SPMs in plasma, a study of rats reported that resolvin synthesis was increased as a result of oral supplementation with DHA for

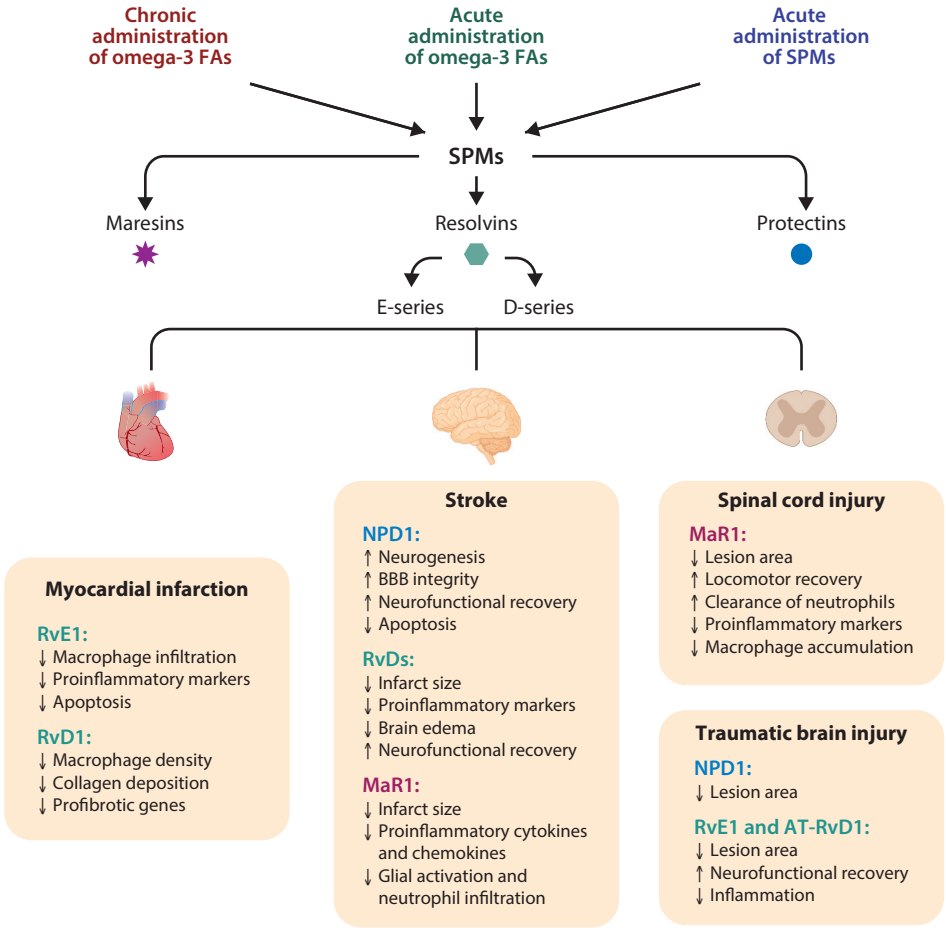

Figure 2

SPMs in heart, brain, and spinal cord injuries. SPMs, classified as protectins, resolvins, and maresins, are produced from the enzymatic oxygenation of EPA and DHA (116). SPM levels can be enhanced by acute or chronic administration of omega-3 FAs. When exogenously administered, SPMs exert protective effects on heart, brain, and spinal cord injuries. In animal models of myocardial infarction, both RvE1 and RvD1 showed cardioprotective effects by reducing infiltration of macrophages and secretion of proinflammatory mediators (65, 75). NPD1, RvDs, and MaR1 also exhibited neuroprotective effects in rodent models of cerebral ischemia. These mediators induced neovascularization, maintained BBB integrity, and promoted cell survival signaling pathways when administered acutely postinjury (12, 140, 149). Recent evidence shows the beneficial effects of SPMs on SCI. Treatment with MaR1 postinjury reduced lesion area and reversed proinflammatory response (50). In TBI, single-dose intralesional administration of NPD1 had brain-tissue-sparing effects (15). Similarly, treatments with RvE1 and AT-RvD1 modulated the inflammatory response to TBI (56). Abbreviations: AT-RvD1, aspirin-triggered resolvin D1; BBB, blood-brain barrier; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; MaR1, maresin 1; NPD1, neuroprotectin D1; RvD and RvE, D- and E-series resolvins; SCI, spinal cord injury; SPM, specialized proresolving mediator; TBI, traumatic brain injury.

8 weeks (92). Likewise, dietary supplementation with omega-3 FAs for 10 weeks enhanced SPM production in the cerebral cortex of aged rats (57). Similar to the findings in animal models, human studies have also demonstrated increased levels of SPMs in blood after supplementation with EPA and DHA (98). Three-week supplementation of 4 g of FO per day (1.4 g/day EPA

and 1.0 g/day DHA) markedly increased concentrations of SPMs (D-series resolvins, RvD2, RvD1, and 17R-RvD1; 18*R*-hydroxy-5*Z*,8*Z*,11*Z*,14*Z*,16*E*-eicosapentaenoic acid (18R/S-HEPE); and 17*S*-hydroxy-4*Z*,7*Z*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid (17R/S-HDHA) in plasma in healthy volunteers (81). Thus, chronic administration of omega-3 FAs elicits cardio- and neuroprotection through activation of several interrelated anti-inflammatory pathways.

RvD: D-series resolvin

2.2. Endothelial Function and Blood Pressure

Endothelial dysfunction, a major CVD risk factor, is induced by increased levels of inflammatory cytokines and adhesion molecules, reduction in nitric oxide production, and changes in the balance between plasminogen activator and plasminogen activator inhibitor-1 (39). Dietary omega-3 FAs may improve endothelial function via modulating endothelial cell membrane fluidity and composition, improving relaxation and constriction of the vessels, and decreasing the secretion of adhesion molecules and inflammatory cytokines (36, 62). Wang et al. (137) reported that omega-3 FAs increased nitric oxide bioavailability in CVD patients, but not in healthy subjects, at a dose ranging from 0.45 to 4.50 g/day over a median of 56 days. Another study assessing impacts of dietary omega-3 FAs on hyperlipidemic, hypertensive, and diabetic populations showed that increased intake of omega-3 FAs over a 1-year period induced a reduction in vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) levels, and this effect was associated with an improvement in endothelial function in peripheral small arteries (87).

These beneficial actions of omega-3 FAs on endothelial function may also lead to a reduction in blood pressure (20). Indeed, dietary FO provided for 12 weeks lowered blood pressure in spontaneously hypertensive rats and improved endothelial function, and these effects were associated with suppression of sphingolipid-dependent vascular contraction (134). In a meta-analysis of 36 RCTs of supplementation with FO in hypertensive and nonhypertensive subjects, systolic and diastolic blood pressure were reduced by an average of 2.3 and 1.5 mm Hg, respectively, following supplementation with omega-3 FAs over approximately 7 weeks with doses ranging from 0.2 to 15 g/day (median 3.7 g/day) (89). Several clinical trials also suggested that EPA and DHA have differential hemodynamic effects. As an example, Mori et al. (91) conducted a study in which overweight men with hyperlipidemia were randomized to receive 4 g/day of either EPA, DHA, or olive oil (as control group) over 6 weeks. Blood pressure and heart rate were reduced in patients receiving DHA, whereas patients receiving EPA did not show a statistically significant effect (91). These findings outline a molecular and clinical basis for understanding the beneficial effects of omega-3 FAs on the vascular system, altering endothelial dysfunction and hypertension.

2.3. Cardiac Arrhythmias

Another property ascribed to omega-3 FAs is the reduction in susceptibility to cardiac arrhythmias (77). Incorporation of these FAs into cardiac membrane phospholipids might influence the production of a variety of eicosanoids and lower vulnerability to arrhythmias, which in turn prevent ventricular fibrillation during myocardial ischemia and reperfusion. Omega-3 FA content in cardiac membrane phospholipids might also increase membrane fluidity; still, there is no evidence that such changes in membrane structure have significant effects on electrophysiology in vivo. In rat vascular smooth muscle cells (A7r5 cell line), treatment with EPA for 7 days inhibited resting intracellular Ca^{2+} concentrations and counteracted the effects of agonists [vasopressin, endothelin-1, and recombinant human platelet-derived growth factor (PDGF)], blocking the increase in intracellular Ca^{2+}. EPA also affected the membrane potential of mitochondria and significantly inhibited PDGF-induced migration of vascular smooth muscle cells (4). McLennan

(85) studied arrhythmias induced by myocardial ischemia in rats fed a diet rich in tuna FO, sunflower oil, or sheep fat for 3 months, and showed that rats fed only the tuna-FO-rich diet were protected from ischemia- and reperfusion-induced ventricular fibrillation. In line with these findings, studies of marmoset monkeys indicated that a diet enriched with tuna FO consumed for 30 months reduced the ventricular fibrillation threshold and lowered the incidence of sustained ventricular fibrillation (86).

Although in vitro and animal studies indicate several pathways whereby omega-3 FAs might exert antiarrhythmic properties, specific mechanisms in vivo are not fully understood, and the effects in humans are still controversial (110). In favor of antiarrhythmic effects of omega-3 FAs, Mozaffarian et al. (94) demonstrated that subjects who consumed fish 1 to 4 times per week showed a 28% lower risk of atrial fibrillation compared with subjects who consumed fish less than once a month. In support of this finding, Nodari et al. (97) conducted a study in which patients with persistent atrial fibrillation were treated with amiodarone and a renin–angiotensin system inhibitor and randomized to 1.7 g of omega-3 FAs per day or placebo starting 4 weeks before electrical cardioversion. After a 1-year follow-up, the omega-3 group maintained sinus rhythm and showed significant reduction in atrial fibrillation recurrence compared with the placebo group (97). Contrary to these findings, in a study by Kowey et al. (71), patients with paroxysmal atrial fibrillation or persistent atrial fibrillation receiving 6.7 g of omega-3 FAs per day for 7 days showed no improvements in cardiac arrhythmias. Similarly, patients with persistent atrial fibrillation and randomized to 2.6 g of omega-3 FAs per day or placebo for 3 weeks prior to cardioversion showed no effect on atrial fibrillation (18).

The heterogeneity of the outcomes on arrhythmic effects in human studies using omega-3 FAs might be due to the selection of varied study populations, different modalities of administration, and possible interactions with concomitant medications and medical devices. It can also be argued that, in the negative studies in which short-term diet or supplements with omega-3 FAs were used, the intervention period did not allow a full incorporation of omega-3 FAs into atrial myocytes.

2.4. Atherosclerosis and Plaque Stabilization

Atherosclerosis, a pathological process in the aorta, coronary arteries, and cerebral arteries, is the major cause of CVD. Lipid deposition is an initial key step in atherogenesis that begins with the entry of lipoproteins [mainly low-density lipoproteins (LDLs)] into the arterial wall. This initiates a proinflammatory cascade that attracts monocytes into the subendothelial space (29, 37). Infiltrated monocytes differentiate into macrophages that take up lipoproteins and become foam cells. These foam cells make up the fatty streak, a precursor of the atherosclerotic plaque (125).

Many studies, including our own, have shown that omega-3 FAs regulate pathways related to atherogenesis (29). Toward a mechanistic understanding of how FAs affect atherogenesis, we (114) have reported that dietary saturated FAs increased arterial lipoprotein lipase (LpL) levels, and this was associated with increased arterial LDL deposition even before the development of atherosclerotic lesions. The potential underlying mechanisms are associated with improved plasma lipid profiles and inhibition of adverse inflammatory responses. Our studies have shown that omega-3 FAs not only markedly decrease local arterial LpL levels but also decrease arterial wall macrophages and inhibit other pathways important in atherosclerotic plaque formation and progression (28, 30).

The vulnerability of atherosclerotic plaque predisposes to plaque erosion or rupture, which causes acute coronary syndrome. EPA and DHA prevent the proliferation and migration of smooth muscle cells, a main step in atherosclerotic plaque formation and progression. Apart from inhibiting plaque progression, omega-3 FAs also prompt plaque stability by reducing macrophage infiltration and releasing matrix metalloproteinases. In support of this, Matsumoto et al. (82)

demonstrated that plaque macrophages isolated from mice fed a FO containing diet for 12 weeks had the lowest expression of ICAM-1 and macrophage scavenger receptors (MSR-A) when compared with plaque macrophages from mice fed a control diet without FO. Similarly, patients awaiting carotid endarterectomy and receiving FO for approximately 40 days before surgery showed plaques with more fibrous cap atheromas, fewer macrophages, and less inflammation (132).

MPTP: mitochondrial permeability transition pore

2.5. Mitochondrial Functionality and Oxidative Stress

There is growing evidence that dietary omega-3 FAs have effects on mitochondrial membrane phospholipids and mitochondrial functionality. Initial studies showed that high intake of FO rich in DHA and EPA prevented age-related decreases of omega-3 FAs in rat cardiac mitochondrial membranes (102). Furthermore, hearts isolated from rats receiving an omega-3 FA diet for 8 weeks showed marked improvement in the recovery of mitochondrial energy metabolism after ischemic injury when compared with hearts from control rats (42). Similarly, dietary supplementation with DHA, but not with EPA, increased omega-3 FA content in rat cardiac mitochondrial phospholipids and improved the tolerance of isolated mitochondria to Ca^{2+}-induced opening of the mitochondrial permeability transition pore (MPTP) (69).

The reduction in the volume of cardiac and cerebral infarction might also be mediated by the antioxidant activity of omega-3 FAs; in fact, they reduce the content of lipid peroxides and activate enzymatic antioxidant defense systems, such as catalase, superoxide dismutase, and glutathione peroxidase, which are responsible for scavenging free radicals (46, 99). A diet enriched with DHA for 12 days counteracted the effects of concussive injury (TBI) on rats and increased superoxide dismutase and Sir2 (a NAD^+-dependent deacetylase) as antioxidant defenses (139). These findings demonstrate that dietary omega-3 FAs provide cardio- and neuroprotective effects that are correlated with changes in mitochondrial membrane composition and improved mitochondrial resistance to oxidative stress.

2.6. Angiogenesis and Neurogenesis

Particularly relevant to stroke, vascular remodeling triggered by the reduction in blood flow is a major endogenous defense in the early phases after ischemic injury; in later stages, revascularization is associated with angiogenesis through endothelial cell proliferation and subsequent formation of new blood vessels (76). This poststroke process greatly prompts brain perfusion and contributes to long-term functional recovery (145). A study conducted by Wang et al. (136) demonstrated that transgenic mice (*fat-1*) overproducing omega-3 FAs had enhanced poststroke revascularization and endogenous angiogenesis, and this occurred in parallel with improved long-term neurofunctional recovery. Similarly, Zhang et al. (144) demonstrated that prolonged administration of FO in rats increased cerebral omega-3 FA levels and offered long-term histological and neurological protection against ischemic brain damage. Such effects were associated with brain revascularization as well as neurogenesis and oligodendrogenesis (144).

The sustained intake of omega-3 FAs likely leads to structural and signaling changes in cell membranes in the spinal cord. Rats fed a diet enriched with omega-3 FAs for 8 weeks before being subjected to SCI exhibited a fundamentally distinct neurolipidome while displaying significantly improved neurofunctional outcome (49). Likewise, supplementation with DHA prior to or post-TBI reduced the impact of experimental injury through similar structural and signaling modulatory processes (8, 139). These findings demonstrate that dietary omega-3 FAs might exert neuroprotection by reducing neuronal and oligodendrocyte loss, facilitating neurogenesis, and thus preserving brain and spinal cord tissue integrity.

3. ACUTE TREATMENT WITH OMEGA-3 FATTY ACIDS

In sharp contrast to oral supplementation, IV administration of omega-3 FAs leads to early incorporation of these FAs into plasma lipids, as well as cell and tissue membranes, within 30 to 60 min, as reported from studies of experimental animal models and humans (25, 26, 105, 118). We suggest that these rapid changes in omega-3 FA membrane content will prove highly effective in settings of acute organ injury. Integration of omega-3 FAs into cell membranes after dietary supplementation requires longer and more complex processes of absorption and occurs over days to weeks. Once reaching the heart, brain, and other tissues, omega-3 FAs are preferentially incorporated into membrane phospholipids or stored as triglycerides. Infusing omega-3 FAs intravenously bypasses more complex routes of delivery and offers possibilities for acute regulatory responses. To examine the effects of chronic versus acute administration, Huang et al. (58) reported that postinjury dietary supplementation with DHA for 1 week in a rat model of SCI, without an acute bolus injection of DHA administered 30 min after injury, was ineffective in showing neuroprotective effects. However, adding the acute DHA IV injection post-SCI to the oral supplementation resulted in a reduction in lipid peroxidation, protein oxidation, and RNA/DNA oxidation in the injured tissue. Also, macrophage recruitment was markedly reduced and neuron and oligodendrocyte survival was substantially increased in DHA-treated rats after injury. Of note, when the dietary intervention was extended to 6 weeks, DHA-injected rats given dietary DHA showed an additional significant reduction compared with those rats receiving only DHA injections. Thus, the optimum improvement conferred by the combined regimen on several parameters suggests that an acute intervention with omega-3 FAs by IV injection rapidly acts on mechanisms related to the early phases of the injury, and dietary omega-3 FAs may affect later repair processes. **Figure 1** describes the major mechanisms related to both chronic and acute treatment with omega-3 FAs, highlighting the differences in the time to onset of action.

Several studies have provided an understanding of the metabolism of IV lipid emulsions as carriers for omega-3 FAs. Pharmacokinetics studies of several animal models examined how IV lipid emulsions of different compositions in omega-3 FAs affect the fatty acyl content of organs and are metabolized in the blood and different tissues (25, 26, 117). In human studies, the use of IV infusion omega-3 FAs has been associated with a reduction in the length of intensive care unit and hospital stays and with improved physiological parameters (21). In support of these findings, a study (16) of patients undergoing cardiac surgery demonstrated that perioperative IV infusions with a triglyceride emulsion rich in both EPA and DHA produced rapid and significant increases of omega-3 FA concentrations in platelet and atrial tissue membranes. Three omega-3 FA infusions (12 and 2 h before, and then immediately after surgery) in these patients induced a decrease in biological and clinical signs of inflammation (16). Additionally, the same group (41) has demonstrated that omega-3 triglyceride emulsions, when intravenously administered in healthy volunteers, significantly reduced heart rate both at rest and during exercise within 24 h, and oral administration of DHA and EPA required 72 h to observe similar results.

Triglyceride lipid emulsions containing mixtures of medium-chain triacylglycerols (MCTs), soybean oil, and FO have been developed for IV infusions (27). With the rapid hydrolysis of MCTs, human and animal studies showed that these emulsions were cleared from blood faster than other omega-3-FA-containing emulsions (133).

Another emulsion containing only MCTs and FO (in a 80:20 wt% ratio) was developed and tested in animals made deficient in omega-3 FAs. Relevant to CVD, the hearts of these omega-3-FA-depleted rats, removed 1 h after the IV bolus administration of the MCT:FO emulsion, showed an improved recovery of functional parameters after ex vivo ischemia/reperfusion when compared with hearts of other omega-3-FA-depleted rats infused either with saline or with

a control emulsion without FO (103). Similar results were obtained in humans in whom bolus injection of the MCT:FO emulsion showed rapid blood clearance and led to substantial incorporation of EPA into white blood cell and platelet phospholipids within the first hour after injection (24–26). Of note, these results indicated that the combination of MCTs and omega-3 FAs in lipid emulsions markedly facilitated the uptake of omega-3 FAs not only in plasma and liver but also in extrahepatic tissues. On the basis of these observations, we speculate that IV administration of omega-3 FAs is likely to be much more effective than oral forms in situations where a rapid effect is desired, such as during an acute inflammatory response or following an ischemic injury.

MCT: medium-chain triacylglycerol

In parallel to findings on dietary supplementation with omega-3 FAs in both clinical and animal studies, many recent studies have demonstrated IV injections of omega-3 lipids as a feasible method for rapid intervention to limit organ injury. Molecular mechanisms associated with acute administration of omega-3 FAs are summarized below and in **Figure 1**.

3.1. Membrane Structure

In several types of acute organ injury, the homeostasis and metabolism of plasma, membrane, and tissue lipids are altered as a result of host responses and inflammatory processes (45). The IV injection of omega-3 FAs offers an attractive strategy to rapidly generate substantial changes in membrane lipid composition, with a shift toward the activation of protective cell signaling pathways and the formation of bioactive lipid mediators. As an example, rabbits subjected to MI and infused with a triglyceride emulsion enriched with omega-3 FAs immediately after the injury showed a rapid incorporation of omega-3 FAs into myocardial membranes, which was strongly associated with cardioprotection as measured by a reduction in infarct size and a decrease in concentration and expression of oxidative stress markers (84). In another study, rats were infused intravenously with radiolabeled DHA over 5 min to achieve steady-state DHA levels in plasma. The results indicated that total phospholipid DHA concentrations in the brain were increased by exogenous DHA within a few hours after the injection, suggesting a rapid uptake of DHA in the brain (31). Thus, increasing the content of omega-3 FAs in brain tissue might also be favorable in the setting of stroke. When an acute injection with a lipid emulsion containing DHA was administered immediately after ischemic brain injury in neonatal mice, a shift in the composition of mitochondrial membranes, with a substantial increase in DHA content, was observed 5 h after the injection. This effect was associated with the neuroprotective effects of DHA, which included marked decreases in infarct size after ischemic injury along with improved neurofunctional outcomes 24 h and 8 weeks after the initial insult (83).

3.2. Cell Death Pathways

Ischemia/reperfusion injury is especially relevant to MI and stroke and is characterized by an initial restriction of blood supply followed by the subsequent restoration of perfusion and concomitant reoxygenation. This is frequently associated with exacerbated tissue injury and a profound inflammatory response as part of the reperfusion injury. Ischemia/reperfusion leads to the activation of cell death programs, including apoptosis, autophagy, and necrosis (66). SCI and TBI also trigger significant tissue damage and tissue loss due to a multitude of mechanisms, which include mainly necrotic and apoptotic cell death processes in the initial phases after the injury (88).

Apoptosis can be divided into two parallel pathways: (*a*) an extrinsic pathway triggered by extracellular signals activating death receptors, and (*b*) an intrinsic pathway initiated by intracellular stress and largely regulated at the mitochondrial outer membrane by pro- and antiapoptotic members of the B cell lymphoma 2 (Bcl-2) family (34). In several ischemic models, omega-3 FAs have exhibited protective effects by promoting antiapoptotic and antioxidant pathways via

Bcl-2, Bcl-xL, and Bcl-2-related protein A1 (Bfl-1/A1) proteins and by attenuating the expression of proapoptotic executers, such as Bax, Bad, and Bid. As a therapeutic approach for MI, acute administration of omega-3 FAs shows cardioprotective effects, in parallel with a modulation of cell death processes, in several animal models. One study showed that treatment with omega-3 FA lipid emulsion immediately postinjury induced a reduction in apoptotic pathways by activating antiapoptotic proteins in the first few hours of reperfusion and preserved the tissue from necrotic damage when compared with saline injection in control mice (147).

RvE: E-series resolvin

NPD1: neuroprotectin D1

Bioactive mediators synthesized from omega-3 FAs, such as resolvins, can also modulate apoptotic pathways (**Figure 2**). Early treatment with resolvin E1 (RvE1) in mice facilitated myocardial recovery from ischemia by decreasing expression of proapoptotic factors in cardiac tissue (68). In experimental models of neurodegenerative disease and brain ischemia/reperfusion damage, neuroprotectin D1 (NPD1), a DHA bioactive mediator, modulates antiapoptotic pathways when administered acutely postinjury (10).

In the earliest study of omega-3 FAs in traumatic CNS injury, which used a model of thoracic hemisection SCI in adult rats, DHA, administered as a single IV bolus 30 min postinjury, induced significant neuroprotection and reduced neuronal and oligodendrocyte loss; this was associated with a reduction in apoptosis, which was still detectable 7 days after SCI (70). TBI induces primary mechanical injury of brain cells and triggers secondary damage, such as inflammation, cell death processes, and oxidative stress. Results obtained with omega-3 FA acute bolus administration show therapeutic potential in TBI. A single IV acute bolus of DHA injected 30 min after injury can induce significant tissue protection and improve neurological outcome in the controlled cortical impact (CCI) model in mice, an experimental model of concussion injury (130), in a way that is reminiscent of the results reported with a single IV bolus of DHA in SCI (58, 70). Multiple intraperitoneal injections of DHA (first injection administered 15 min after TBI and then repeated daily for up to 7 days) significantly improved hippocampal autophagy flux and cognitive function in CCI in rats, in parallel with inhibition of neuronal apoptosis and neuroprotective effects (141). These studies indicate that acute injection of omega-3 FAs is highly effective in protecting heart, brain, and spinal cord by activating cell survival programs and limiting the extent of injury.

3.3. Transcriptional Regulation

The cardio- and neuroprotective effects of omega-3 FAs include enhancing the expression of several nuclear transcription factors, such as PPARs, which play a role in cytoprotection after injury. PPARγ is a transcription factor largely involved in lipid metabolism, promoting free FA uptake and triglyceride accumulation (55). The exact roles of PPARγ regulation in the heart and in the ischemia/reperfusion scenario have been under discussion. Cardiomyocyte-restricted PPARγ knockout mice develop cardiac hypertrophy, although they present no changes in lipid metabolism genes (43). In contrast, murine cardiac PPARγ overexpression leads to dilated cardiomyopathy, accumulation of triglycerides, and increased free FA uptake (122). Another study also showed that acute injection with an omega-3 FA lipid emulsion during reperfusion after ischemic insult in mice downregulated PPARγ protein expression in the heart, and this effect was markedly associated with substantial cardioprotective effects induced by omega-3 FAs (147).

Acute treatment with omega-3 FAs also enhances the expression of different nuclear factors in animal models of stroke and brain injury. Luo et al. (78) demonstrated that, in rats subjected to cerebral microinfarcts, acute administration of omega-3 FAs postinjury significantly inhibited the activation of receptor-interacting serine/threonine protein kinase 1 and its downstream apoptosis-associated proteins, and this effect was associated with reduced infarct size and preserved neurofunctional outcomes as evidenced by improved learning and short-term memory.

Following nuclear translocation of NF-κB, cells release large numbers of inflammatory cytokines, initiating a cascade amplification of inflammatory responses. Activation of the NF-κB pathway is mainly associated with high mobility group box 1 (HMGB1), which translocates from the nucleus to the cytosol and activates necrotic pathways. In TBI or SCI, NF-κB and HMGB1 are actively involved in the inflammatory response (101, 124). Omega-3 FA acute injection inhibited HMGB1 nucleocytoplasmic translocation/extracellular secretion and alleviated HMGB1-mediated activation of the NF-κB pathway following TBI-induced microglial activation, thus blocking the subsequent inflammatory response (32). Also, intracerebroventricular injection of maresin 1 (MaR1), a DHA-derived SPM, immediately after ischemic brain injury in rats reduced infarct size and neurological defects, alleviated NF-κB p65 activation and nuclear translocation, and, in turn, reduced proinflammatory factor (IL-1, IL-6, and TNF-α) levels (140). Protective effects of MaR1 in a mouse model of SCI, in which the mediator was injected intravenously 1 h after SCI, with the injection repeated daily until day 7, have also been reported (50). Thus, acute injection of omega-3 FAs exerts cardio- and neuroprotective effects through interdependent mechanisms, which involve the regulation of transcription factors and gene expression.

MaR1: maresin 1

3.4. Mitochondrial Functionality and Oxidative Stress

Mitochondrial dynamics play key roles in the pathophysiology of cardiovascular and CNS diseases. Of note, mitochondrial dysfunction represents a common early pathological event in brain and heart injuries. A major marker for mitochondrial dysfunction is the MPTP, which is a nonspecific voltage-dependent special protein complex spanning the mitochondrial outer membrane that controls mitochondrial permeability. The MPTP is in an off state in physiological conditions, whereas during ischemia the MPTP is open; this is triggered by both calcium overload in the mitochondrial matrix and elevated oxidative stress. The opening of the MPTP leads to an increase in mitochondrial permeability, allowing solutes such as water, large molecules, and ions to enter freely into the mitochondrial matrix, and this in turn causes mitochondrial swelling, outer membrane rupture, and abundant release of ROS. Moreover, this cascade also causes loss of membrane potential, further decreased ATP levels, and enhanced intracellular calcium concentration, with a subsequent activation of cell death signaling pathways (135, 146).

In an ex vivo murine model of ischemic injury, hearts perfused with DHA or its mediator, 19,20-epoxydocosapentaenoic acid (19,20-EDP), displayed a marked reduction in mitochondrial Drp-1 and Mfn-2 as well as maintained Opa-1 levels, three key regulatory proteins in mitochondrial fusion. DHA and 19,20-EDP modulated the activities of both cytosolic thioredoxin 1 (Trx-1) and mitochondrial Trx-2 as regulators of hypoxia-inducible factor 1, and these effects markedly preserved mitochondrial integrity (35).

Acute administration of omega-3 FAs can also preserve mitochondrial functionality. For example, in neonatal rats subjected to hypoxic-ischemic injury, acute injection of DHA 10 min preceding the hypoxia phase maintained mitochondrial inner membrane integrity and transmembrane potential and improved synaptic markers (3). In another study reported by our group (83), neonatal mice were injected with a lipid emulsion containing either DHA or EPA immediately after ischemic brain injury. Isolated brain mitochondria from the mice treated with DHA were resistant to Ca^{2+}-induced membrane permeabilization and showed remarkable decreases in ROS production within 30 min to a few hours after the insult, whereas EPA acute injection failed to attenuate mitochondrial dysfunction (83). In addition, Zhang et al. (143) reported that DHA, when administered intravenously 1 h after induction of subarachnoid hemorrhage in mice, provided neuroprotection and preservation of mitochondrial functions. In summary, acute treatment with

omega-3 FAs translates to cardio- and neuroprotective effects, with maintenance of mitochondrial functionality playing a major role, especially in the initial postinjury period.

3.5. Inflammatory Pathways and Omega-3-Derived Specialized Proresolving Mediators

Insufficient or inadequate resolution of inflammation after ischemic injury leads to chronic inflammation that causes greater tissue damage, impaired tissue remodeling, and inappropriate tissue healing. The resolution of inflammation is an active process regulated in part by a superfamily of lipid mediators, SPMs, derived from omega-3 FAs as described above (116). The importance of SPMs in the resolution of inflammation is evident in many acute pathological conditions, and when their endogenous production is insufficient, delayed, or even absent, exogenous administration of SPMs can reverse proinflammatory processes and mediate tissue protection in both heart and CNS (115) (**Figure 2**).

With regard to cardiac injury, early acute injection of RvE1 post-MI suppressed the infiltration of macrophages and the secretion of proinflammatory cytokines in injured mouse hearts, protecting cardiomyocytes against apoptosis in the peri-infarct zones. In contrast, delayed treatment with RvE1 1 week after MI failed to reduce neovascularization in the peri-infarct zones. This indicates that RvE1 may serve as an early therapeutic agent for acute MI (75). The role of RvD1 in post-MI inflammation has also been investigated. Using coronary artery ligation as an experimental model for MI, Kain et al. (65) showed that administration of RvD1 3 h postinjury reduced macrophage density, increased macrophage-mediated clearance of necrotic cells, and decreased collagen deposition, thereby reducing post-MI fibrosis and stabilizing the extracellular matrix (65).

Acute administration of NPD1 (intracerebral or IV) in animal models of brain injuries induces neuroprotection, neovascularization, and cell survival signaling pathways (10, 12, 15). In addition, RvD2, when administered in rats immediately after cerebral ischemic injury, reduced infarct size, inflammatory response, brain edema, and neurological dysfunction (149).

To examine the effect of two resolvin molecules on TBI, researchers injured mice using a midline fluid percussion injury model and injected them with either RvE1 or aspirin-triggered RvD1 (AT-RvD1) daily for 7 consecutive days beginning 3 days prior to TBI. Treatment with RvE1 ameliorated posttraumatic inflammatory response to TBI, although independently of improvement in motor and cognitive outcomes as shown in mice treated with only AT-RvD1 (56).

Of note, exogenous administration of omega-3 FAs can also affect SPM profiles and facilitate resolution processes. Thus, DHA, when acutely injected as a triglyceride emulsion in neonatal mice, induced an increase in SPM levels (NPD1 and RvDs) in brain 2 h after ischemic brain injury (83). Similarly, after CCI in mice, DHA, injected intravenously 30 min postinjury, modified SPM levels in the injured hemisphere 3 h post-CCI (131). With bioactivity in picomolar to nanomolar concentrations, SPMs appear particularly well suited as therapeutics in acute settings of heart, brain, and spinal cord injuries.

Recent publications indicate that omega-3 FAs injected as an acute bolus can also modulate the early inflammatory response posttrauma. Paterniti et al. (100) have shown that in mice with compression SCI, DHA administered intravenously 30 min after injury reduced the tissue inflammatory processes within 24 h through mechanisms likely to involve PPARα, as the neuroprotective effects were attenuated in PPARα knockout mice. Hall et al. (54) reported a decrease in neutrophil infiltration and a decrease in C-reactive protein following IV administration of DHA in a rat model of SCI, whereas EPA did not induce such effects. These studies support the hypothesis that acute administration of omega-3 FAs exerts beneficial effects in part through the activation

of anti-inflammatory responses and the biosynthesis of bioactive mediators after injuries in heart, brain, and spinal cord.

4. CLINICAL TRANSLATION OF CHRONIC AND ACUTE TREATMENT WITH OMEGA-3 FATTY ACIDS IN CARDIOVASCULAR AND CENTRAL NERVOUS SYSTEM INJURIES

4.1. Recent Omega-3 Fatty Acid Clinical Outcome Trials

Results from cardiovascular outcome trials involving omega-3 FAs have been inconsistent. Whereas earlier trials showed promising results, several subsequent trials failed to demonstrate efficacy of omega-3 FAs, as reported in a recent systemic review by Abdelhamid et al. (1). The authors concluded that EPA and DHA have little or no effect on mortality or cardiovascular health. In **Table 1**, we summarize the CVD outcomes from the major omega-3 FA clinical trials.

Table 1 Clinical trials of omega-3 fatty acids and cardiovascular diseases[a]

Study	Study design	Intervention	Follow-up	Outcome
Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) Prevenzione	Secondary prevention RCT	882 mg of EPA and DHA per day	3.5 years	Positive: 15–20% reduction in mortality and cardiovascular events
Diet and Reinfarction Trial (DART)	Secondary prevention RCT	200–400 g of fish per week	2 years	Positive: 29% reduction in mortality
DART-2	Secondary prevention RCT	2 servings of fish per week or 3 capsules of FO per day	3–9 years	Negative: higher cardiac mortality and sudden cardiac death
Japan EPA Lipid Intervention Study (JELIS)	Primary and secondary prevention RCT	1.8 g of EPA per day	4.6 years	Positive: benefits in secondary prevention with 19% reduction in coronary events
GISSI–Heart Failure (HF)	Secondary prevention RCT	840 mg of EPA and DHA per day	3.9 years	Positive: 9% reduction in mortality and 8% reduction in hospitalizations
Alpha Omega	Secondary prevention RCT	226 mg of EPA and 150 mg of DHA per day	3.4 years	Negative: no significant differences
OMEGA	Secondary prevention RCT	460 mg of EPA and 380 mg of DHA per day	1 year	Negative: no significant differences
Supplementation with Folate, Vitamin B_6 and B_{12} and/or Omega-3 Fatty Acids (SU.FOL.OM3)	Secondary prevention RCT	600 mg of EPA and DHA per day	4.7 years	Negative: no significant differences
Outcome Reduction with Initial Glargine Intervention (ORIGIN)	Secondary prevention RCT	465 mg of EPA and 375 mg of DHA per day	6.2 years	Negative: no significant differences

(*Continued*)

Table 1 (*Continued*)

Study	Study design	Intervention	Follow-up	Outcome
Randomized Trial to Assess Efficacy of PUFA for the Maintenance of Sinus Rhythm in Persistent Atrial Fibrillation (FORWARD)	Secondary prevention RCT	1 g of omega-3 FAs per day	1 year	Negative: no significant differences
A Study of Cardiovascular Events in Diabetes (ASCEND)	Primary prevention RCT	1 g of omega-3 FAs per day	7.4 years	Negative: no significant differences
Vitamin D and Omega-3 Trial (VITAL)	Primary prevention RCT	1 g of omega-3 FAs per day	5.3 years	No significant differences in primary outcomes Significant differences in secondary outcomes (MI)
Statin Residual Risk Reduction with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia (STRENGTH)	Primary prevention RCT	4 g of omega-3-carboxylic acids per day	3–5 years	Terminated 1/2020 by Data and Safety Monitoring Board for low likelihood of demonstrating benefit
Reduction of Cardiovascular Events with EPA-Intervention Trial (REDUCE-IT)	Primary prevention RCT	4 g of EPA per day	4.9 years	Positive: ~25% reduction in major cardiovascular events (MI and stroke)

[a]All trials are referenced in Abdelhamid et al. (1). The ASCEND, VITAL, STRENGTH and REDUCE-IT trials are referenced in the ASCEND Study Collaborative Group (5), Manson et al. (80), Nicholls et al. (96), and Bhatt et al. (17), respectively.
Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FA, fatty acid; FO, fish oil; MI, myocardial infarction; RCT, random controlled trial.

Recent publications of four large clinical outcome trials—A Study of Cardiovascular Events in Diabetes (ASCEND), Vitamin D and Omega-3 Trial (VITAL), Statin Residual Risk Reduction with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia (STRENGTH), and Reduction of Cardiovascular Events with EPA-Intervention Trial (REDUCE-IT)—have provided new perspectives on the use of omega-3 FA therapies in patients with CVD. ASCEND examined whether a daily supplement of omega-3 FAs could decrease cardiovascular events in diabetic patients without known CVD. Compared with the placebo group that received olive oil capsules, the group receiving a daily supplement of 1 g of omega-3 FAs did not show a reduction in the risk of serious vascular events in diabetics without known CVD (5). VITAL evaluated the effect of a daily supplement of vitamin D3 (2,000 IU) and FO (1 g) on the primary prevention of cancer and CVD. After a follow-up of over 5 years, groups receiving omega-3 FA supplements did not show a lower incidence in the primary end points of major cardiovascular events (a composite of MI, stroke, or death from cardiovascular causes) and invasive cancer of any type, compared with placebo groups (80). However, the group receiving omega-3 FA supplements showed several statistically significant effects on secondary outcomes, including a 28% reduced risk for heart attacks, with a 50% reduced risk for fatal heart attacks, and a 17% reduced risk for total coronary heart disease events.

STRENGTH, a large-scale global cardiovascular outcomes trial, evaluated the safety and efficacy of Epanova (omega-3-carboxylic acids, containing EPA and DHA) compared with placebo, both in combination with standard-of-care statins, on decreasing the risk of major adverse cardiovascular events in patients at high risk of CVD (96). However, the phase 3 STRENGTH trial was discontinued because the ongoing data showed no beneficial effects.

In the face of these somewhat negative results, REDUCE-IT has stimulated a potential revival of new approaches to omega-3 FA supplement use in CVD—in particular, a focus on using only EPA, but in contrast with the Japan EPA Lipid Intervention Study (JELIS) and other trials, in which doses of EPA were much higher (17). REDUCE-IT selected high-risk patients with hypertriglyceridemia on statin therapy who were randomized to receive either 4 g of ethyl EPA daily or a placebo. The follow-up period was 4.9 years. There were significant reductions in the first, subsequent, and total ischemic events for each individual component of the composite primary end points (61 versus 89 per 1,000 patient years for icosapent ethyl versus placebo, respectively). These results strongly suggest that the benefits of ethyl EPA on different ischemic end points (e.g., coronary and cerebral, fatal and nonfatal events) depend not only on the ability of this compound to lower triglyceride levels but very likely on other mechanisms of action that may work synergistically and contribute to the observed outcomes.

Despite the large number of studies evaluating marine-derived omega-3 FA interventions for heart disease, there is only low-quality and mixed evidence for their effects on stroke populations. Bouzan et al. (19) evaluated a dose–response relationship between fish consumption and stroke risk, and the results indicated a small relative risk reduction after fish consumption but not for no fish consumption. JELIS showed that EPA had no preventive effect on stroke events in the primary prevention subgroup (128). Of interest, REDUCE-IT reported that the use of 2 g of ethyl EPA twice daily among patients with high triglyceride levels was superior to placebo in reducing cardiovascular death, with a significant reduction of 20% in stroke recurrence. A recent systematic review and meta-analysis showed mild to moderate association between fish consumption and lowered risk for cerebrovascular diseases (33). Some of these studies reported a reduction in risk (~26%) after oral treatment with omega-3 FAs in patients with transient ischemic attack; however, the number of participants was limited, showing wide confidence intervals (33). Alvarez Campano et al. (2) reported that subgrouping by intervention type, cotherapy, trial duration, or dose did not provide significant differences in stroke outcomes. Adequately powered studies of hemorrhagic stroke and subarachnoid hemorrhage are also needed, considering that the only data available were from a small pilot study (113) in which patients received parenteral treatment with omega-3 FAs and showed improved primary outcomes 90 days after subarachnoid hemorrhage. Although animal studies suggest important effects of acute administration of omega-3 FAs as neuroprotectants, no trials have specifically addressed their use in acute ischemic stroke (2). Thus, future clinical trials could focus on effective therapeutic windows, optimal dosages, and routes of administration (ingestion or injection) of omega-3 FAs.

As mentioned above, most primary prevention trials and recent secondary prevention trials in CVD have failed to replicate the results of the earlier studies of omega-3 FAs. Such discrepancies between study results may be explained by variations in the background baseline of omega-3 FA intake, the heterogeneity in study designs, the timing of the initiation of omega-3 FA supplements, a low-dose EPA or DHA supplement in many trials, insufficient length of follow-up to see benefits, inadequate controls, underpowered statistical analyses, inaccurate reporting of individual responsiveness and adherence, or confounding factors introduced by cotherapies (40). At the same time, CVD treatments have also progressed, and the standards of control conditions have evolved.

We suggest a few questions to address when proceeding with new clinical trials:

- Does protection depend on the daily dose of omega-3 FAs?
- Do effects differ between the sources of dietary and supplemental omega-3 FAs?
- Which route of administration should be selected?

The chemical form in which the FAs are delivered and the interaction with food might affect the degree of absorption of omega-3 FAs. Of note, ethyl ester omega-3 FA absorption is low when

consumed in a fasting state, whereas absorption is considerably higher when consumed with a high-fat meal and intermediate with a low-fat meal (79). An important strategy based on these observations might be to identify a chemical form that can facilitate the absorption of omega-3 FAs. For example, in a study conducted by Raatz et al. (108), 10 subjects consumed either 4 g of an emulsified FO supplement or four 1-g capsules of triglyceride FO, and plasma phospholipid FAs were measured periodically over 48 h. The group receiving the emulsified FO supplement showed a substantial increase in total omega-3 phospholipid FA levels in plasma when compared with the group receiving the capsules. Thus, coingestion of emulsifiers with FO improves the digestion and absorption of omega-3 FAs. Furthermore, comparing different routes of administration will allow researchers to better evaluate the ability of these omega-3 FAs to lower cardiovascular and CNS disease risk. On the basis of the results obtained in REDUCE-IT, it is also important to explore whether EPA is sufficient to get maximal cardiovascular benefit, whether DHA alone is equally as effective, or whether a specific combination of EPA and DHA at higher doses would be even more bioactive than either FA alone. In parallel, better delineation of the similarities and differences in molecular pathways affected by the bioactive omega-3 FAs are needed as adjuncts in the design of future clinical trials.

4.2. Future Potential of Omega-3 Fatty Acid Acute Therapeutics

With the increasing interest in strategies to rapidly enhance delivery of EPA and DHA to specific tissues and organs, acute IV injections of omega-3 FAs and perhaps rapid-delivery oral formulations may offer novel approaches to postinjury therapeutics. Numerous oral omega-3 FA products, which vary widely in the amount of EPA and DHA they provide, are listed in the US National Institutes of Health Dietary Supplement Label Database (48, 60). Recently, absorption-enhancing technologies that increase EPA and DHA bioavailability in omega-3 FA oral products have been developed (107).

Until 2016, the only IV lipid emulsion approved for use in the United States was composed of 100% soybean oil. However, alternative IV lipid emulsion blends containing soybean oil, olive oil, FO, and/or MCTs have been used since the 1990s in Europe, South America, and Asia for adult and pediatric patients requiring parenteral nutrition. In 2016, the US Federal Drug Administration (FDA) approved the first composite mixed-oil omega-3 FA IV lipid emulsions for nutritional support in adult patients: a four-oil lipid emulsion composed of soybean oil (30%), MCTs (30%), olive oil (25%), and FO (15%), known as SMOFlipid® (Fresenius Kabi). Currently, three FO-containing lipid emulsions are commercially available for use in parenteral nutrition: Lipoplus® (known in some countries as Lipidem®; B Braun), a 50:40:10 (by volume) mixture of MCTs, soybean oil, and FO; SMOFlipid; and Omegaven® (Fresenius Kabi), which is 100% FO (21). Omegaven is currently approved in the United States and elsewhere as a lipid emulsion for infants with liver disease associated with IV nutrition (47). Of note, all available IV lipid emulsions containing omega-3 FAs are approved for nutrition support over hours or days but not for acute bolus injection. An alternative to administering omega-3 FA emulsions intravenously might be to use albumin as carrier. Exogenous administration (continuous infusion over 3 min) of DHA bound to human albumin, in fact, has been previously shown to be neuroprotective in an adult rodent model of stroke (13). However, albumin has the disadvantage that, at high doses, it could expand intravascular volume and induce pulmonary edema (53). Also, direct infusion of FAs with albumin at high doses could lead to encephalopathy and hepatotoxicity (120).

All the orally prescribed omega-3 FA products have been generally well tolerated in clinical trials and have well-established tolerability profiles. In the case of lipid emulsions, all omega-3-FA-enriched emulsions currently approved for nutrition support in total parenteral nutrition have undergone safety and toxicity studies in animals and humans. However, safety studies of acute

bolus injection are still needed, although one study using bolus administration of a MCT:FO emulsion in human volunteers reported no adverse effects (24). FO and its effect on bleeding have been the subject of debate, yet there is no evidence to date that suggests any concerns regarding the supplementation with omega-3 FAs and the occurrence of adverse bleeding in patients. In a clinical trial described above assessing the efficacy of an omega-3 lipid emulsion infused over 4 h as acute treatment in patients with subarachnoid hemorrhage, no side effects in fact were reported (113).

Our review emphasizes the importance of time delays in managing cardiovascular and CNS diseases and proposes that acute administration of omega-3 FAs, although sharing similar protective mechanisms with chronic administration, might have the advantage of providing faster bioavailability of these lipids. For this reason, a potential strategy to be considered in the design of future clinical trials would be to combine therapeutic regimens of omega-3 FA oral supplements with omega-3 FA injections, leading to increased and sustained protection after acute injury. For instance, in animal models of stroke or SCI the combination of these two different routes of administration (oral and IV) offered synergistic neuroprotection when compared with each therapy alone (58, 63). This enhanced effect might be attributed to potentially complementary mechanisms. Indeed, the acute IV treatment with omega-3 FAs could activate molecular pathways in the early minutes or hours of the injury and then oral supplementation with omega-3 FAs would contribute to postinjury tissue healing and repair and preservation of organ functionality.

As described above, in animal models of SCI and stroke, DHA showed more neuroprotection than EPA. In contrast, when translated to clinical trials, as reported by JELIS and REDUCE-IT, using only long-term administration of EPA was effective in decreasing CVD risk. Thus, there is a need to not only define the similarities and differences in the underlying mechanisms linked with chronic versus acute administration of omega-3 FAs but also characterize specific molecular effects of EPA versus DHA.

DISCLOSURE STATEMENT

R.J.D. is a founding scientist and member of the scientific advisory board of DeckTherapeutics, Inc., a company developing diglyceride lipid emulsions to prevent tissue death after ischemic organ injuries. R.J.D. is an inventor on Columbia University–assigned patents on omega-3-rich diglyceride emulsions as potential agents for cytoprotection of different organs after ischemic injury. None of these patents or plans for DeckTherapeutics involve the potential use of compounds discussed in this review as a neuroprotectant. Y.A.C. is founder and director of Nutrition Lipid Developments, SPRL, a company that aims to develop novel lipid emulsions for therapeutic applications and that is a holder of European Patent 02748862 and US Patent 2004/0247693. Y.A.C. received consultancy fees from Beijing Sciecure Pharmaceuticals, Inc. The other authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

H.Z. wrote the initial draft of this article. H.Z., C.L.C., and R.J.D. reviewed and contributed to all drafts. All authors contributed to the revisions of the final version of this article.

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