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# Fish Oil Metabolites: Translating Promising Findings from Bench to bedside to Reduce Cardiovascular Disease

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## Abstract

Cardiovascular disease is an inflammatory process and the leading cause of death in the United States. Novel omega-3 derived potent lipid mediators, termed resolvins and protectins, have been identified as major pathophysiologic players in the resolution phase of the inflammatory response. Potent lipid mediators offer tremendous metabolic and pathophysiologic insights in regard to the risk and treatment of cardiovascular disease. In this review, resolvins and protectins are described and analyzed as accelerators of discovery via their potential role as biomarkers for research and clinical decision making in cardiovascular disease. Specific barriers relating to biomarker validation, laboratory methods, and improvement of risk models are introduced and discussed. Potential therapeutic impacts in cardiovascular disease are also mentioned with special consideration for cost-saving implications with respect to dietary fish oil as an alternative to resolvin and protectin treatment. Given the high tolerability of fish oil supplements and previously described benefits of omega-3 fatty acid intake in cardiovascular disease, we conclude that resolvins and protectins are set to soon take center stage as future biomarkers and well-tolerated therapies for cardiovascular disease.

**Keywords:** Omega-3 fatty acids (N3 Fatty Acids); Resolvin; Protectin; Fish oil; Eicosapentaenoic acid (EPA); Docosahexaenoic acid (DHA); Biomarker; Cardiovascular disease (CVD)

## Introduction

A great deal is known about the four stages of inflammation and the cellular response that occurs in tissues, but much less has been elucidated regarding active resolution of the inflammatory process via naturally occurring molecules [1]. Given the high throughput (omic) laboratory methods that have become available for identifying a great variety of proteins, carbohydrates, and lipids, the ability to detect compounds that exert such inflammation-resolving actions has been greatly enhanced [2]. Resolvins and protectins are lipid mediators that have recently emerged as important factors in the active resolution of inflammatory processes in vivo and have been the target of study in the last several years. This becomes very important for the prevention and treatment of cardiovascular diseases given the role that inflammation plays in these disorders. Current evidence has demonstrated that these lipid mediators are produced in humans from omega 3 poly-unsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA), found in marine oil sources. The accessibility, affordability, and lack of adverse effects related to fish oil supplementation make these potent lipid mediators favorable for much more extensive studies in humans with or without cardiovascular disease. However, several challenges in the translational pathway exist that are described in this review. Other manuscripts have paid a great deal of attention to the potentially promising biochemical effects of the potent lipid mediators discussed in this article, and thus we are focusing on the issues that make application of these mediators to humans particularly challenging.

## Preclinical Data

To-date, the resolvins and protectins have been studied almost exclusively *in vitro* and within animal studies, with some human studies demonstrating temporal relationships in relation to cardiovascular surgery intervention [3,4]. The essential omega 3 fatty acids from fish oil supplements EPA and DHA have been shown to be the building blocks of resolvins and protectins. There are two classes of resolvins, the D-series that derive from DHA, and the E-series that derive from EPA (See Figure 1). Although the focus of our paper is the resolvins and protectin molecules, this figure also points out that lipoxins derived from the omega-6 arachidonic acid also have demonstrated inflammation resolving effects.

In contrast to the actions of products of omega-3 fatty acids, arachidonic acid (an omega 6 polyunsaturated fatty acid) yields eicosanoids, such as prostaglandings and leukotrienes, that play a role in the propagation and sustainability of the initial inflammatory process [5]. However, once the inflammatory process proceeds to the initial resolution stages, arachidonic acid and its leukotrienes products can be converted to lipoxins which stop leukocyte recruitment and help to promote generation of lipid mediators such as resolvins and protectins from EPA and DHA [6].

The D-series resolvins produced from DHA work to resolve inflammation by preventing tumor necrosis factor alpha (TNF-alpha) from making pro-inflammatory cytokines that would otherwise continue to sustain neutrophil infiltration [4,7-9]. Compared to the E-series resolvins, a specific group of D-series resolvins are aspirin-

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triggered after acetylation of the cyclooxygenase 2 (COX2) enzyme by aspirin and its interaction with DHA.

The E-series resolvins include two forms, resolvin E1 (RvE1) and resolvin E2 (RvE2). RvE1 works to resolve inflammation through effects on responding neutrophils. Specifically, RvE1 stops the diapedesis of polymorphonuclear leukocytes (PMNs) to the site of inflammation, blocks the PMNs' response to inflammatory cytokines, as well as enhances the destruction of already-present PMNs via phagocytosis by macrophages [10]. RvE1 also upregulates the expression of CCR5 receptors on PMNs, which blocks the cell from producing pro-inflammatory chemokines and induces the neutrophil to undergo apoptosis and make it a target to be phagocytized by macrophages [11]. It has recently been discovered that RvE1 can regulate and stop adenosine diphosphate activation of human platelets, adding yet another facet to the pro-resolution armamentarium [12]. There is less known about the specific activity of RvE2, but it has been shown that this lipid mediator is also produced by neutrophils and acts in a similar way to RvE1. Current evidence has demonstrated that even though the two forms of E-series resolvins function in approximately the same way, they must have their own receptors as there is an additive effect when administered in conjunction [10]. Figure 2 outlines the inflammation resolving effects of the families of potent lipid mediators of resolvins, protectins, and lipoxin A4.

Protectins are lipid mediators derived from DHA. They function as anti-inflammatory molecules by blocking the activation and migration of neutrophils, as well as suppressing the production of inflammatory cytokines [13]. Like the resolvins, protectins enhance the expression of chemokine receptor type 5 (CCR5) ligands on neutrophils and inhibit NF-κB induction halting neutrophil transmigration [4,14,15]. Although the protectin receptor is still unknown, like RvE2, there is a combined effect with RvE1 indicating the receptors are distinct [13]. These data strongly support the potent effects of EPA and DHA metabolites on the active resolution of inflammation. Potential health benefits of these EPA and DHA metabolites derived from animal model studies are outlined nicely in two recent reviews by Serhan [4,16].

## Clinical Implications

Inflammation is a major factor in a multitude of diseases, making the potential benefits of pro-resolution lipid mediators widespread. The vast majority of the studies have been in animal models including mice, rats, and rabbits [16,17], but there is a fair amount of potential overlap with human disease processes that could benefit from pro-resolution molecules. RvE1 has been linked to resolution of pulmonary processes including asthma and acute lung injury, colitis, inflammatory pain, peritonitis, periodontitis and retinopathy [17,18]. RvE2 is less widely studied, but has been proven to resolve inflammation from peritonitis in murine models with a similar potency level to RvE1 and likely has effects on the many other human diseases in which RvE1 is potentially protective [18,19]. D-series resolvins also work to decrease inflammation related to peritonitis and retinopathy, as well as to help protect murine kidney tissue from reperfusion injuries [20]. Protectins help resolve peritonitis, retinopathy, asthma hyper-responsiveness, and kidney reperfusion injuries, though they appear to be especially important in neural tissue by protecting glial tissues during ischemic stroke [13]. Protectin concentrations have been studied in humans, and low levels have been associated with asthma exacerbation and Alzheimer's disease [4,21].

To this date, there are no large studies that have measured the Omega-3 Index (a measure of red blood cell membrane EPA and DHA) and correlated this level to resolvins or protectins concentrations in humans or in animal models. The Omega-3 Index is an interesting risk marker for cardiovascular disease that continues to gain traction for acceptance in research and clinical settings [22].

There are, however, preliminary data suggesting that the ingestion of EPA and aspirin does appropriately lead to an increase in 18S-hydroxyeicosapentaenoate (18S-HEPE), which is a RvE1 precursor. Without aspirin or EPA supplementation, typical values of this compound were measured to be 24.0 +/- 5 pg/ml in healthy subjects. Three hours after supplementation with ASA 81 mg and EPA 1 gram, 18S-HEPE levels increased to 56.6 +/- 19 pg/ml compared to 27.7 +/- 7.8 pg/ml with EPA supplementation alone [23]. Thus, evidence suggests that omega-3 fatty acid supplementation alone might not be the answer for "reducing inflammation" but, rather, the combination of aspirin with omega-3 fatty acids, which leads to the generation of inflammation-resolving compounds, creating a milieu that favors the resolution of the inflammatory process.

## Specific Cardiovascular Implications

As atherosclerosis has been recognized as an inflammatory process, it can be surmised that pro-resolution lipid mediators can have a

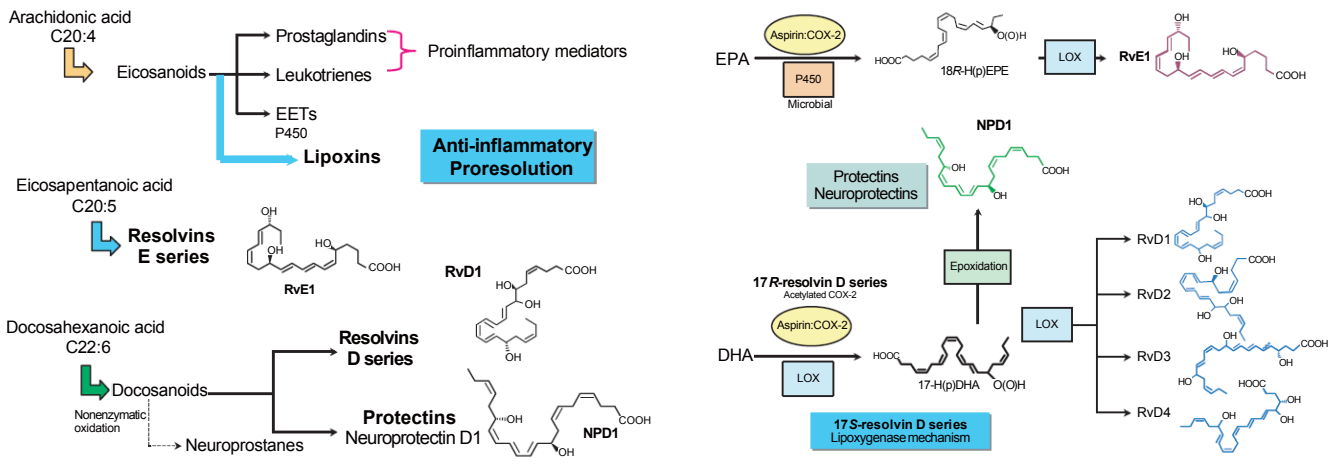


**Figure 1:** Schematic diagram of the synthesis of polyunsaturated omega-3 and omega-6 derived lipid mediators.

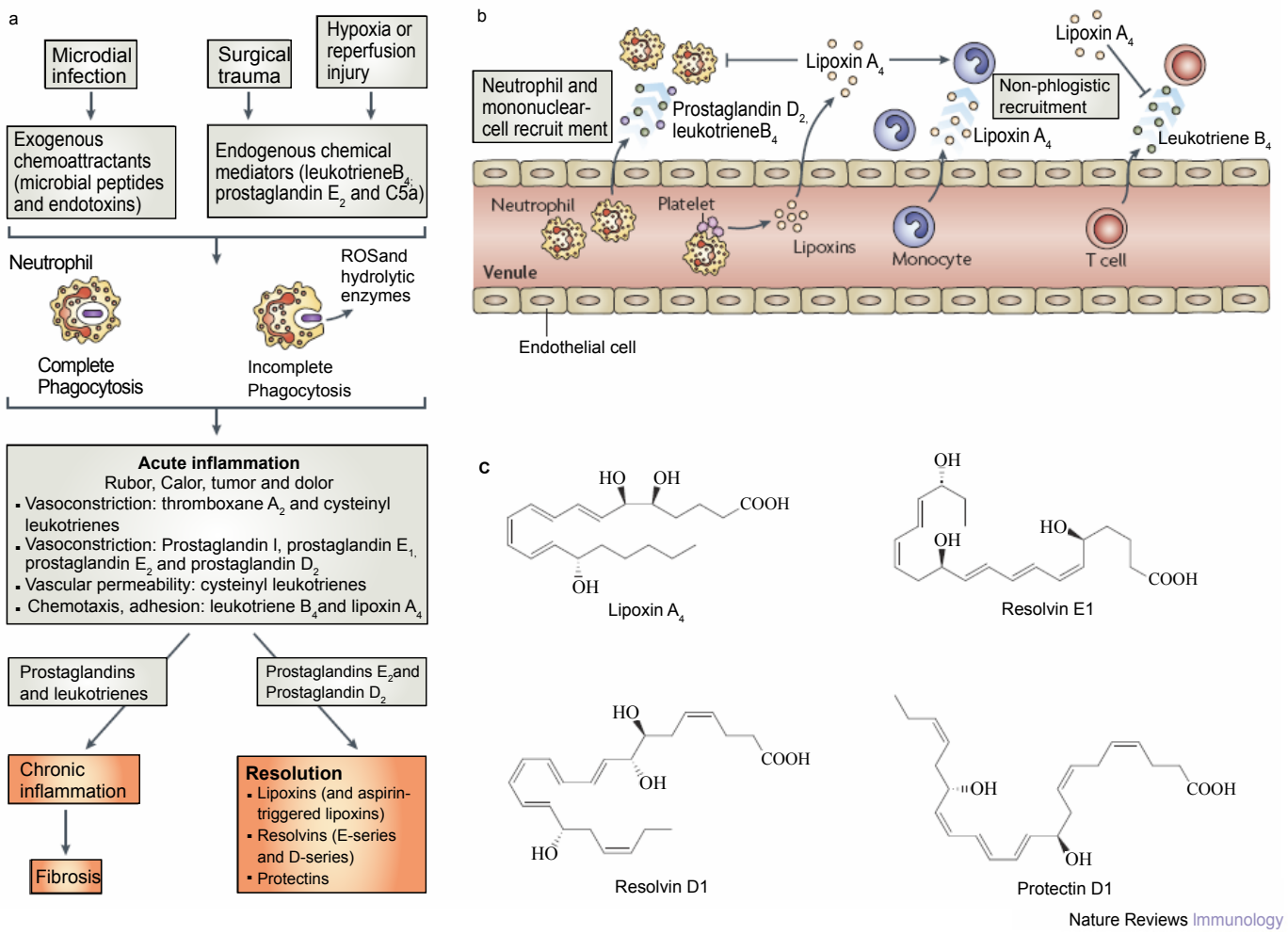


**Figure 2:** Decision paths in acute inflammation: resolution or chronic inflammation and the roles of endogenous chemical mediators.

similar benefit in cardiovascular diseases. The burden of cardiovascular disease throughout the world is massive, so it is especially important to pursue the study of direct effects of resolvins and protectins on vascular inflammation because it would be very feasible to start patients who are at a variety of risk levels for cardiovascular events on fish oil supplements. Fredman et al. [12] have shown in human blood that RvE1 suppresses ADP-dependent activation of platelets, preventing platelet aggregation and therefore negating an essential process that leads to cardiovascular events. There has been very interesting research in rat cardiomyocytes showing that RvE1 prevents leukocyte diapedesis following ischemia, thus preventing reperfusion injury and decreasing the resulting ischemic zone size [24]. In murine models, expression of 12/15 lipoxygenase was associated with resolvin biosynthesis, atheroprotection, and a delay of atherosclerosis development underscoring the temporal relationship of pro-resolution cascades [25].

Since the vast majority of studies regarding resolvins and protectins have been carried out using isolated human cells and animal models, the translation of such data to humans is facilitated by determining whether temporal profiles between pro-inflammatory mediators and pro-resolving mediators are demonstrable in vivo. Very recent data in patients undergoing abdominal aortic aneurysm surgery have demonstrated that temporal relationships for local-acting peptides (e.g., VEGF, IL-10, TGF(β)) and lipid mediators (leukotrienes and resolvins) exist [3]. In addition, profiles obtained for these patients placed them into two groups based on their temporal profile: one group consistent with a pro-inflammatory and another with a resolving profile. These facts may have clinical implications for patients with a high risk of cardiovascular disease or in patients for whom the diagnosis has already been established, as co-administration of aspirin and fish oil supplements could potentially have additive effects on the protection of vessels and cardiomyocytes where "pro-resolution failure" is suspected. These translational metabolomic profiles demonstrate temporal relationships between local mediators in humans relevant in inflammation resolution.

## Challenges in Translating Knowledge to Improve Human Cardiovascular Health

As the studies discussed have been primarily categorized as basic research, it is important to leverage translational sciences to allow for application to the bedside care of patients. In order to proceed with clinical studies, several challenges must be met. The use of lipidomics technologies for the measurement of potent lipid mediators in the midst of clinical research or the assessment of a correlation between

molecule concentration and human disease is certainly potentially very promising. However, this capability assumes consistency in the laboratory methods used (i.e. measurement technologies, tissue lipid extraction, training of staff) and in the process of obtaining and preserving blood or other tissue in the midst of a study or clinical scenario. Without consistencies existing, the comparison of data from one research or clinical laboratory with data from other laboratories will be potentially difficult [26]. These issues become particularly important because resolvins and protectins tend to work as autocoids locally [4], being produced and degenerated rapidly and thus may not be optimal as standard blood biomarkers. Thus, the potential for blood handling/processing, venipuncture, and subject drugs, as examples, to alter mediator concentrations exist [27]. An increase in concentrations could potentially occur due to the activation of cyclooxygenase by platelet activation or inadvertent cooling while blood is stored in the Vacutainer used during collection.

Before advancement in the use of resolvins and protectins as markers of inflammation resolution, there needs to be a reliable laboratory method to measure accurate levels of the lipid mediator. Laboratory personnel and equipment such as mass spectrometers will have to be available for these studies, and it will need to be understood how to extrapolate from daughter metabolite molecules shown on mass spectroscopy to the parent molecules from the sample. Assays have to be produced and studies demonstrating sensitivities, specificities, minimum sample size, and source have to be performed. Normal ranges have to be established with interindividual variability taken into account. The cost of running the assays will also be an important consideration in the utility of these tests in translational studies and feasibility for public use. All of the above traits will have to be reproducible, and the methods must be proven by validation studies.

A major benefit of the ability to measure potent lipid mediators in humans is that such levels can be correlated with identification of risk when examining the presence or absence of disease and associated outcomes. These molecular epidemiology measurements can be very important for both observational studies as well as clinical trials. Many criteria for this ability will need to be met prior to demonstration of actual feasibility. The sensitivity, specificity, and positive and negative predictability of the tests are particularly relevant [28]. The cost, sample amount required, technology required, and the ability required by a technician performing the test, would best be specified prior to a laboratory using the lipidomic methods for measuring the molecules. Success in the use of blood values for clinical research is also dependent on expertise in biostatistics, molecular biology, toxicology, and laboratory science [28]. This expertise can be very useful prior to beginning a clinical study and during the conduct of the research because only with its implementation will the assay's feasibility and predictability be validated. Given the vast data detectable using lipidomics methodologies, it is also important for investigators to think clearly about which molecules are most important for analysis as well as the bioinformatical and biostatistical approaches that make the most sense for implementation. Implicit in these issues is the fact that many hypothesis-generating and hypothesis-testing outcomes are viable for inclusion in most studies involving lipidomics and other high throughput laboratory approaches. It is crucial for those who conduct clinical research to realize the potential drawbacks of blood measurements before using them in a research project. This not only involves adequate data supporting the validity of the lipidomic measurement method, but also strong rationale for this approach, including a realistic hypothesis that the results will lead to improved health.

When the laboratory methodologies have been standardized and there is a reliable way to measure the presence and level of resolvins or protectins in a given sample, further research can ensue to test the validity of using this lipid mediator as a biomarker. Inflammation is a very complicated process involving numerous chemical mediators and it occurs throughout the body in different ways. When researchers become able to track levels of resolvins and protectins, observational and clinical studies can provide correlation between lipid mediator levels and cardiovascular end points. However, it will be admittedly difficult to differentiate among the many forms of inflammation occurring at one time, as well as the acuity or chronicity of the process. It will also be very important that levels of these mediators demonstrate correlation with health and disease independent of standard known variables that have demonstrated strong associations with health status.

When considering establishment of a biomarker, it has to be determined whether the measured mediator is a risk factor that is active in a pathway leading to disease, or is a risk marker that is a sign of the disease [29]. This differentiation is very critical for understanding whether alteration of concentrations can improve health. Regarding the investigation of resolvins and protectins as pro-resolution molecules, biomarker levels could actually be an indicator of improvement or worsening of the inflammatory process related to atherosclerosis. If levels of resolvins and protectins are followed and increase with fish oil administration, would this indicate the efficacy of the supplementation and/or improve the cardiovascular status of the patient being studied? If a correlation like this could be established, there could be huge economic implications. Fish oil supplementations are quite inexpensive, and if they can be used to help prevent the morbidities and mortalities that result from cardiovascular disease, a large amount of health care dollars could potentially be saved.

Obvious implications of a focus on the health benefits of EPA and DHA ingestion versus treatment with resolvins or protectin are important to consider and investigate given the many factors including cost, safety, and public health implications that are affected by which option is selected. The increasing focus on reducing the medical care costs and the increasing focus of the National Institutes of Health on issues of cost-effectiveness and the translation of knowledge from basic science research into the clinical and community settings needs to be given adequate consideration. The average cost of an over-the-counter EPA/DHA supplement currently is quite minimal and Lovaza®, currently a brand-name medication, may become available as a generic in September of 2012 when GlaxoSmithKline's exclusive rights are set to expire. It is also possible that EPA/DHA ingestion is most beneficial for human health when ingested within seafood, not as a supplemental drug. The fact that EPA and DHA are the sources of resolvins and protectins implies that there may be human physiological factors, including cyclooxygenase enzyme activity variation, that optimally determine the amounts of these metabolites that are generated and exist in tissue. Thus, it may be that the ingestion of EPA/DHA is more conducive to homeostasis than ingestion or another source of exposure of metabolites, particularly in the setting of optimal diet, exercise, other lifestyle characteristics, and the ingestion of drugs (including aspirin) that can increase the production of resolvins and protectins [30]. Alternatively, the doses and purities of metabolites that are becoming available for research may be significantly more effective for a series of health issues than can be achieved with EPA/DHA ingestion. Much of any debate of whether EPA/DHA or metabolite administration is superior for any disorder will also be answered by the stability of each when formalized as components of pharmacologic agents. Ultimately, many clinical trials will be required to sort out these questions.

## Potential Benefits of Translating Knowledge to Improve Human Cardiovascular Health

It is well known that dyslipidemia and resultant atherosclerosis result to a great degree from the imbalance of the lipid metabolites in the affected organism [27]. Lipidomic studies can quantify the precise chemical constituents of lipidomes (including the distribution of resolvins and protectins), identify lipid cellular distribution, and describe their biochemical mechanisms, interactions, and dynamics [31]. Such information could be useful prognostically for patients based on their molecular lipid profiles. Resolvins and protectins are end points of biological systems, and because of the tight regulation supported by current data [32], they are possible prime biomarker candidates.

Lipidomics in combination with clinical samples and biobank materials is increasingly being used to address the many unmet needs of disease diagnostics [27]. Resolvins and protectins may certainly be excellent candidates for companion diagnostics in the pharmaceutical arena, which is moving increasingly toward specialized therapeutic models. As the pharmaceutical industry moves from its past approaches to more specialist therapies, it will be increasingly important to be able to cater to a specialist group by identifying the correct patients who should receive a medication at the correct time and correct dose. Companion diagnostics, a crucial component of personalized medicine, are highly useful tools because they can provide not only the ability to identify those patients who will benefit from the therapy (i.e., the responders), but to also identify those who are not tolerant of the treatment [33]. The measurement of resolvins and protectins using lipidomics methodologies could help to address clinical and research patient selection and stratification as well as treatment efficacy and safety while taking drugs. The promising data produced to date suggest that the measurement of resolvins and protectins can aid in discerning phenotype information that will help us to better understand many gene-gene, gene-environment, and gene-protein interactions that are involved in the development of atherosclerosis.

Another very promising domain existing for the measurement of resolvins and protectins in humans is their potential therapeutic applications [4]. Aspirin acetylates COX and blocks the metabolism of arachidonic acid into a variety of proinflammatory and thrombosis-enhancing mediators including thromboxane [34-37] . The omega 3 fatty acids EPA and DHA compete with arachidonic acid for the same COX pathways and can also inhibit the production of inflammatory mediators. Thus, aspirin and omega 3 fatty acids work in parallel to shift the fatty acid metabolic balance toward a less inflammatory milieu. The production of resolvins and protectins from EPA and DHA may also be driving this process as it has been shown that RvE1 downregulates platelet and leukocyte activation [12,38], well known components of cardiovascular disease. The anti-inflammatory effects of resolvins and protectins have implications for numerous cardiovascular diseases for which few effective therapies are available including stroke [17,39], renal ischemia-reperfusion injury [4,20] and retinopathy [7,17]. Although the encouraging data on these disorders has been generated from animal research, the likelihood that these lipid mediators will have similar effects in humans should be high considering some previously published data regarding EPA and DHA in humans [40-42].

As with most translational first-in-human investigational drugs, identifying the safest starting dose for early phase clinical trials could be a challenge. The Food and Drug Administration has established guidance documentation to aide in this process [43]. Ongoing early phase trials of synthetic and natural resolvins in conditions such as asthma, dry eyes, and inflammatory bowel disease are currently underway; however, information as to doses of these compounds has not been made publically available [44]. In one particular study of renal reperfusion injury with 23 to 28 gram mice, intravenous resolvin and protectin doses ranged from 3 to 35 μg/mouse [20]. Using this study as an example, anticipated human equivalent doses should range from 0.01 to 0.1 mg/kg. However, in another study investigating the effects of RvE1 in asthma mouse models, a reduction in leukocyte bronchoalveolar lavage fluid, airway mucus, and airway hyperactivity was pronounced with intravenous doses ranging from 50 to 200 ng/mouse [45]. At same time, other successful studies have used intrathecal RvE1 and RvD1 at doses of 10 ng/mouse in order to investigate their effects in acute and persistent pain [46]. These studies seem to indicate that careful consideration to the disease process in question should take place with particular attention not only to dosing but also to the most appropriate route of administration.

A particularly affirming characteristic of the potential human benefits of treating and preventing cardiovascular disease with resolvin and protectin lipid mediators is that they are biological molecules, derived from omega 3 fatty acids, and are generated naturally in the average human. They are lipids and thus act as cell signaling, highly protective particles, which modulate more than one (and often several) tissues via pleotrophic effects [4]. These facts distinguish them from many industrially produced drugs and what have been traditionally considered the primary biological therapeutic agents, which tend to have more limited and specific effects. An important result of this distinguishing nature of lipid biologics is that they may not be prone to the concerning fact that “biosimilars”, or protein biopharmaceuticals, often differ from traditional chemically produced equivalent drugs due to their size and complexity of the active substance, and their manufacturing process [47]. In contrast to classical generics, “biosimilars” are proteins manufactured via biological processes such as recombinant DNA, and are not identical to their originator products. This concern has lead governments and the FDA to conclude that they should not be brought to market using the protocols applied to generics. This is due largely to implications of their manufacturing, as well as efficacy and safety controls of biosimilars when compared to their small-molecule generic counterparts [48,49], which could lead to serious health implications [47]. Thus, generic versions of biologics are not authorized in the European Union or the U.S. through the more straightforward procedures allowed for small molecule generics. All of these issues are not important for the generation of potent lipid mediators via the supplementation of EPA and DHA from fish oil or algae-derived DHA because these are already authorized for human consumption in the U.S. either through seafood consumption or supplements.

In order for these potent lipid mediators to serve as biomarkers in quantitative risk assessment measurement, internal and external validity must be shown in the studies that include them [50]. The obstacles mentioned earlier related to lab accuracy and sample handling apply in this setting. Fortunately, over the past several decades, inflammation research has given us a glimpse for understanding the basic mechanisms by which inflammation is propagated and terminated. Pathophysiologic mechanisms of various disease processes already support the use of these molecules as potential markers of uncontrolled inflammation and disease progression. The elements of measurement validity (construct, criterion, and content validity) seem to be well aligned for the success of these biomarkers in disease prognosis. These potent lipid mediators relate to the underlying phenomena, e.g. inflammatory disease development; their absence

could predict the persistence of an early inflammatory process and the development of chronic processes that ultimately lead to the disease state; and lastly, these markers show promising capability to already differentiate pro-inflammatory vs pro-resolution profiles that correlate well with other biomarkers of underlying disease or lack thereof.

Establishing potent lipid mediators as diagnostic markers of disease will be more challenging than utilizing them for risk assessment. While lack of these compounds is sensitive to active pro-inflammatory disease or chronically deranged inflammatory processes, they are unlikely to be disease specific. Evidence suggests that these markers play a role in a myriad of conditions where the underlying pathophysiology is inflammation. In some cases, because of the high prevalence of some of the diseases in question, e.g. cardiovascular disease and asthma, they could have strong predictive value when subjects are evaluated for these medical conditions. Potent lipid mediators are most likely to serve as adjuncts in disease diagnosis, but not sole-identifiers of a specific disease. Moreover, complex diseases like cardiovascular disease have multiple inciting pathways where multiple biomarkers play a role in disease progression. In these instances where multiple pathways are at play, the disease process itself likely will affect the presence and measurement of the pro-resolution mediator. Nevertheless, once the appropriate studies are performed relating measurement of these potent lipid mediators with health outcomes, measurement of these biomarkers can serve to diagnose inflammatory states that do merit clinical intervention.

## Conclusions

Lipid mediators that act as inflammation pro-resolution molecules have the potential of benefiting patients with a vast array of health conditions. Since *in vitro* and animal studies have shown positive effects of resolvins and protectins, and considering the ease of the intervention to add fish oil supplementation to patients' supplement and/or medication regimens, trustworthy and expedient translational research needs to occur to allow progress toward implementing the pro-resolution effects of these molecules in many human diseases and syndromes. The numerous obstacles interfering with the establishment of measurement validity and biomarker characteristics of lipid mediators need to be surmounted when aiming to use them for risk assessment and as therapeutic agents. It is likely that not only will fish oil and its metabolites have promising therapeutic effects on current cardiovascular disease; they could also have a role in preventing the underlying inflammation that has implications for other diseases.

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Information on Re-engineering the Clinical Research Enterprise can be obtained from http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp.

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