Novel n-3 Docosapentaneoic Acid-derived Pro-resolving Mediators Are Vasculoprotective and Mediate the Actions of Statins in Controlling Inflammation

Jesmond Dalli[1,2], Kimberly Pistorius[1] and Mary E Walker[1].

[1]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, UK, EC1M 6BQ

[2]Centre for Inflammation and Therapeutic Innovation, Queen Mary University of London, London, UK.

Contact:

Dr Jesmond Dalli Ph.D, William Harvey Research Institute, John Vane Science Centre,

Charterhouse Square, London. EC1M 6BQ. E-mail: j.dalli@qmul.ac.uk

Tel: +44 (0) 207 882 8263

Abstract:

Inflammation is a fundamentally protective process that protects the host from invading pathogens and it is central in the repair and regeneration of damaged tissue. However when uncontrolled, the overzealous response leads to tissue damage and malaise. Indeed, this process is now appreciated to be at the center of many chronic diseases including vascular disease and arthritis. Studies investigating the mechanisms through which acute inflammation is actively turned off to allow the tissue to regain function demonstrated that in inflammatory exudates the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are enzymatically converted to bioactive mediators. These mediators carry distinct structures actively, reprogram the inflammatory reaction to promote its termination, counter-regulate the production of pro-inflammatory mediators and regulate leukocyte trafficking as well as phenotype. Recently we found that n-3 docosapentaenoic acid, which was until then only regarded as a biosynthetic intermediate in the formation of DHA from EPA, is also converted to structurally distinct bioactive mediators that reprogram the host immune response. In the present review we will discuss the evidence underpinning the biological actions of these molecules in particular as they pertain to the vascular system.

Introduction

Success in the evolution of multicellular organisms was, at least in part, reliant on the development of a system to repair and regenerate damaged tissues as well as to defend the organism from invading microbial pathogens [1]. This defense system is embodied in the inflammatory process which when self-limited is fundamentally protective and coordinates both the killing and disposal of invading pathogens as well as the repair and regeneration of damaged tissues [2]. However, when this process becomes dysregulated it leads to disease [3-7]. Pioneering studies investigating mechanisms that regulate the termination of inflammation uncovered a new genus of mediators produced via the stereoselective conversion of essential fatty acids, termed as specialized pro-resolving mediators (SPM) [8]. This superfamily includes the arachidonic acid (AA)-derived lipoxins, the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) resolvins [9], and the DHA-derived protectins [10] and maresins [11]. These mediators share select biological actions that include i) limiting neutrophil recruitment to the site of inflammation, ii) they counter-regulate the production of pro-inflammatory mediators including prostaglandins, leukotrienes, cytokines and chemokines (e.g. Tumor necrosis factor alpha), iii) promote the uptake and killing of bacteria and iv) increase the uptake and clearance of apoptotic cells [3-6,8]. In addition, each of the mediators exerts unique biological actions for example, the EPA-derived resolvin E1 regulates platelet activation [12], the DHA-derived protectins regulate viral replication [13] and the DHA-derived maresins promote tissue regeneration [3,14].

SPM exert their potent biological actions via the activation of specific G-protein coupled receptors which include the Lipoxin A_4 receptor (ALX/FPR2), GPR32/DRV1, GPR18/DRV2 and the chemerin receptor ChemR23/ERV1 [15]. In addition to activating cognate receptors, these protective mediators also regulate the onset and propagation of inflammatory responses by acting as partial agonists or antagonists to receptors of inflammatory mediators including BLT-1, the Leukotriene (LT)B_4 (5S,12R-dihydroxy- 6Z,8E,10E,14Z- eicosatetraenoic acid) receptor [16],

and cysLT1 the receptor for LTC_4 (5S-hydroxy-6R-(S-glutathionyl)-7E,9E,11Z,14Z-eicosatetraenoic acid) and LTD_4(5S-hydroxy-6R-(S-cysteinylglycinyl)-7E,9E,11Z,14Z-eicosatetraenoic acid) [16]. Studies investigating mechanisms involved in the onset and propagation of inflammatory disorders indicate defects in the production of the pro-resolving mediators, their enhanced further metabolism or defects in the signaling via their cognate receptors are linked with disease onset and/or progression [3-6,8].

In mammals, the essential fatty acid alpha-linolenic acid (9Z, 12Z, 15Z-octadecatrienoic acid; ALA) is enzymatically elongated and desaturated to produce EPA and subsequently to DHA with n-3 docosapentaenoic acid (7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid; n-3 DPA) being the biosynthetic intermediate in this process [17-19]. n-3 DPA contains a 22-carbon chain with 5 double bonds and differs from DHA as it lacks a cis-double bond at carbon 4, which, although a small change, provides its own specific biologically relevant actions [20,19]. These actions have been identified in a variety of mammalian tissues, including plasma, brain, retina and heart. Genome-wide association studies in humans uncovered a correlation between increases in peripheral blood n-3 DPA concentrations and single nucleotide polymorphisms in the gene encoding for the fatty acid elongase 2 (ELOVL2)[21]. We recently found that, in addition to EPA and DHA, n-3 DPA is substrate for conversion to novel families of bioactive mediators [20]. The aim of the present review is to discuss the actions of these novel families of mediators in regulating key leukocyte responses.

n-3 DPA-derived SPM are novel resolution agonists

Events that occur in the early phases of an inflammatory reaction are suggested to determine whether the response is self-limited or perpetuates and becomes chronic [22]. Assessment of events occurring within the circulation during acute self-limited inflammation demonstrated that the concentrations of n-3 DPA were rapidly upregulated during acute inflammation, to an extent that was comparable to essential fatty acids that are involved in the

biosynthesis of lipid mediators, including arachidonic acid and DHA. Using a systematic approach coupling structure elucidation with functional readouts we found that endogenous n-3 DPA is converted to bioactive mediators in both mice and human leukocytes that carried pro-resolving properties [20]. These mediators are congenerous with DHA products, namely D-series resolvins ($RvD_{n\text{-}3\ DPA}$), protectins ($PD_{n\text{-}3\ DPA}$) and maresins ($MaR_{n\text{-}3\ DPA}$), with unique stereochemistries [20]. In self resolving exudates, the production of these molecules was temporally regulated where for example $RvD1_{n\text{-}3\ DPA}$ (7S,8R,17S-trihydroxy-9E,11E,13Z,15E,19Z -docosapentaenoic acid) and $PD2_{n\text{-}3\ DPA}$ (16,17-dihydroxy-7Z,10,13, 14,19Z- docosapentaenoic acid) displayed a bi-phasic profile, reaching a maximum during peak neutrophil infiltration and late into resolution. $PD1_{n\text{-}3\ DPA}$ (10R,17S-dihydroxy-7Z,11E,13E,15Z,19Z-docosapentaenoic acid), $MaR2_{n\text{-}3\ DPA}$ (13,14-dihydroxy-7Z,9,11, 16Z,19Z-docosapentaenoic acid) and $MaR3_{n\text{-}3\ DPA}$ (4, 21-dihydroxy-7Z,10Z,12E,16Z,19Z-docosapentaenoic acid) levels were each found to reach a maximum at the 4h interval and gradually decreased over the next 20 h. The peak in exudate $RvD2_{n\text{-}3\ DPA}$ (7S,16,17S-trihydroxy-8,10Z,12,14,19Z-docosapentaenoic acid) levels coincided with the onset of resolution (the point where PMN levels reach ~50 of T_{max}). $RvD5_{n\text{-}3\ DPA}$ (7S,17S-dihydroxy-4Z,8,10Z,13Z,15,19Z-docosahexaenoic acid) levels were found to gradually increase over the course of inflammation-resolution, with a maximum being reached late in the resolution phase. The n-3 DPA product corresponding to $MaR1_{n\text{-}3\ DPA}$ (7S,14S-dihydroxy-8E, 10E, 12Z, 16Z, 19Z-docosapentaenoic acid) gave levels that were elevated in the peritoneum of naive mice, where upon challenge with zymosan these levels drastically decreased. $MaR1_{n\text{-}3\ DPA}$ accumulated late during resolution [20]. Of note each of these molecules displayed leukocyte directed actions whereby incubation of human neutrophils with $RvD5_{n\text{-}3\ DPA}$ or $PD1_{n\text{-}3\ DPA}$ markedly reduced neutrophil adhesion to TNF-α activated endothelial cells and chemotaxis towards IL-8, to a similar extent as the DHA-derived RvD2 (7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z- docosahexaenoic acid) [23,20,24]. These mediators regulate the expression of adhesion molecules on peripheral blood leukocytes and platelets including the

expression of CD11b on neutrophils and monocytes as well as CD62P and CD63 on platelets [25]. Furthermore, they also regulate the formation of leukocyte-platelet heterotypic aggregates in both human and mouse peripheral blood. The biological actions of these molecules extend beyond the regulation of mechanisms in leukocyte trafficking. Indeed, n-3 DPA-derived SPMs also regulate the uptake of apoptotic cells by macrophages, a key biological action in the resolution of inflammation, with increases in macrophage efferocytosis of up to 70% at doses as low as 1nM [23,20]. These mediators also display endothelial directed actions, counteracting the TNF-α- mediated upregulation of adhesion molecules, such as Intercellular Adhesion Molecule 1 (ICAM-1/CD54), on endothelial cells [20].

Diurnal regulation of $RvD_{n\text{-}3\ DPA}$ controls vascular leukocyte and platelet activation

Circadian mechanisms are at the heart of a number of physiological functions, including leukocyte and platelet responses [26,27]. Disturbances to various aspects of these fundamental mechanisms are thought to be responsible for many of the diseases that afflict modern societies, including cardiovascular and metabolic disorders [26-28]. These conditions are characterized by a dysregulated inflammatory response, although the exact mechanisms that underlie this inflammatory state remain of interest. Recent studies demonstrate that the production of $RvD_{n\text{-}3\ DPA}$ are diurnally regulated in the peripheral blood of healthy volunteers [25] (Figure 1). Multivariate analysis of plasma lipid mediator profiles demonstrated a diurnal shift in plasma LM-SPM concentrations with a leftward shift in LM-SPM clusters from morning to evening profiles. This shift was associated with an increase in the amounts of n-3 DPA derived mediators, including $RvD1_{n\text{-}3\ DPA}$ and $RvD5_{n\text{-}3\ DPA}$ from the evening (18:00 h) to morning intervals (7:00 and 9:00 h). These diurnal changes in peripheral blood $RvD_{n\text{-}3\ DPA}$ concentrations were abrogated in mice lacking the main orchestrator of the molecular clock, BMAL1 in myeloid cells. Of note, the fluctuations in plasma $RvD_{n\text{-}3\ DPA}$ were associated with a regulation of leukocyte and platelet activation that reaches a maximum between 7:00 and 9:00h coincident with an increase

in $RvD_{n\text{-}3\ DPA}$ concentrations. The production of these mediators was found to be under the control of acetylcholine (ACh), with peripheral blood concentrations of this neurotransmitter also reaching a maximum during the early hours of the morning (i.e. 7:00h). Furthermore, incubation of whole blood with ACh increased $RvD_{n\text{-}3\ DPA}$ concentrations, including $RvD2_{n\text{-}3\ DPA}$, under both static and flow conditions.

Assessment of the production of these mediators in patients with cardiovascular disease (CVD) demonstrated significant decreases in plasma $RvD_{n\text{-}3\ DPA}$ concentrations and a marked impairment in their diurnal regulation when compared with healthy volunteers. Flow cytometric analysis of peripheral blood leukocyte from patients with CVD demonstrated increases in the expression of CD11b on both neutrophils and monocytes when compared with healthy volunteers. This was coupled with increases in platelet–neutrophil and platelet–monocyte aggregates in peripheral blood from patients with CVD [25]. In addition, we found a significant relationship between peripheral blood $RvD_{n\text{-}3\ DPA}$ concentration and leukocyte and platelet activation, as demonstrated by a negative correlation between $RvD_{n\text{-}3\ DPA}$ and neutrophil CD41, monocyte CD41, and platelet CD63 and CD42b expression. Investigations into mechanisms that lead to the downregulation of peripheral blood $RvD_{n\text{-}3\ DPA}$ mediators in patients with CVD demonstrated a link between peripheral blood adenosine concentrations and the activity of one of the $RvD_{n\text{-}3\ DPA}$ biosynthetic enzymes, ALOX5. Adenosine, via the activation of the A2a receptor, downregulates the activity of ALOX5 [29], and in CVD patients we found that this nucleoside was upregulated in peripheral blood. The role of adenosine in downregulating $RvD_{n\text{-}3\ DPA}$ concentrations was further underscored by experiments where peripheral blood from CVD patients were incubated with adenosine deaminase, that lead to a significant restoration of peripheral blood $RvD_{n\text{-}3\ DPA}$ concentrations [25].

These findings lead us to propose $RvD_{n\text{-}3\ DPA}$ as endogenous protective signals that control physiological platelet and leukocyte activation. This is further supported by observations made in Apolipoprotein E deficient mice ($ApoE^{-/-}$) mice fed a western diet. Treatment of these

mice with $RvD5_{n\text{-}3\ DPA}$ reduced platelet-leukocytes aggregates *in vivo* and modulated vascular lipid mediator profiles reducing concentrations of the pro-thrombogenic mediator Thromboxane (Tx) A_2 (measured as its metaboliteTxB_2) and upregulating the formation of pro-resolving mediators including MaR1 (7S,14S-dihydroxy-4Z, 8E, 10E, 12Z, 16Z, 19Z-docosahexaenoic acid) and aspirin triggered (AT)-LXA_4 (5S,6R,15R-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid). Furthermore $RvD5_{n\text{-}3\ DPA}$ also decreased early aortic lesions in $ApoE^{-/-}$ mice. The present findings are in line with published findings demonstrating an altered production of vascular DHA-derived SPMs including RvD2 and MaR1 and impaired resolution responses in the pathogenesis of atherosclerosis [30,31]. Together these findings demonstrate that alterations in the diurnal regulation of vascular $RvD_{n\text{-}3\ DPA}$ may occur early in the pathogenesis of cardiovascular diseases that results in vascular inflammation and impaired biosynthesis of DHA derived SPM.

$PD_{n\text{-}3\ DPA}$ regulates macrophage phenotype and function during monocyte to macrophage differentiation

It is now well appreciated that in chronic inflammatory conditions, such as atherosclerosis and rheumatoid arthritis, monocytes play a central role in the initiation, propagation and termination of inflammation [32,33]. Upon recruitment to the site of inflammation, these cells either differentiate to inflammatory macrophages that propagate the inflammatory response or to resolution phase macrophages that promote the termination of inflammation and restitution of tissue function. Studies investigating mechanism that regulate the differentiation of macrophages demonstrate a role for the $PD_{n\text{-}3\ DPA}$ pathway in regulating the phenotype and function of monocyte-derived macrophages [34] (Figure 2). Using a total organic synthetic approach coupled with lipid mediator profiling, human ALOX15 and ALOX15B were identified as the enzymes that catalyse the first two steps in the $PD_{n\text{-}3\ DPA}$ biosynthetic pathway yielding an allylic epoxide. Using total organic synthesis we established the absolute

stereochemistry of this epoxide as 16*S*,17*S*-epoxy-7*Z*,10*Z*,12*E*,14*E*,19*Z*-docosapentaenoic acid [34,35]. This intermediate was in turn converted to $PD1_{n\text{-}3\ DPA}$ and $PD2_{n\text{-}3\ DPA}$ by different epoxide hydrolase enzymes, where in human cells epoxide hydrolase 2 (EPHX2) was found to catalyse the conversion of 16*S*,17*S*-e$PD_{n\text{-}3\ DPA}$ to $PD2_{n\text{-}3\ DPA.}$ Experiments investigating the expression of the $PD_{n\text{-}3\ DPA}$ biosynthetic pathway during monocyte to macrophage differentiation found the expression of all three enzymes was upregulated during monocyte to macrophage differentiation. Of note, ALOX15 expression was higher in M2 differentiated cells, whereas the expression of ALOX15B was higher in M1 cells [34].

Pharmacological inhibition and genetic deletion of ALOX15 enzymes in monocytes led to phenotypic and functional changes in monocyte-derived macrophages. In cells where ALOX15 was inhibited there was a downregulation of several lineage markers including CD206, CD163 and CD64 and a shift in macrophage phenotype [34]. This downregulation in phagocytic receptors was of functional consequence since inhibition of the $PD_{n\text{-}3\ DPA}$ biosynthetic pathway also significantly downregulated the ability of human macrophages to uptake apoptotic cells, a key pro-resolving action [36,37]. Of note, this alteration in macrophage phenotype and function was recovered with the reconstitution of components within the $PD_{n\text{-}3\ DPA}$ pathway. Incubation of human monocyte-derived macrophages with either $PD1_{n\text{-}3\ DPA}$ or 16S, 17S-epoxy-$PD_{n\text{-}3\ DPA}$ led to a restoration of several phagocytic receptors and increased macrophage efferocytosis. Furthermore, administration of $PD1_{n\text{-}3\ DPA}$ to ALOX15 deficient mice also restored the phenotype and efferocytic activity of macrophages *in vivo* [34]. Thus, these findings identify the $PD_{n\text{-}3\ DPA}$ pathway as a component in the monocyte-to-macrophage differentiation program that regulates their phenotype and function.

RvTs are produced during the early stages of acute inflammation and temper host immune responses

Recent studies have described and characterised a new family of bioactive mediators produced from n-3 DPA. This new family of four resolvins is termed the 13-series resolvins (RvT) given that all four molecules display potent host protective actions and carry a hydroxyl group on carbon 13 [38]. RvTs are biosynthesized from n-3 DPA in a process that requires both endothelial cell COX-2 and neutrophil lipoxygenase (ALOX) activity. The role for ALOX enzymes in the biosynthesis of RvT was established using heavy oxygen incorporation [38] with the identity of the ALOX enzymes catalyzing this reaction remaining of interest.

During acute inflammation, cytokines such as TNF-α and IL-1β are released, activating endothelial cells that upregulate the expression of COX-2. Endothelial COX-2 converts n-3 DPA to 13R-hydro(peroxy)-docosa-7Z,10Z,14E,16Z,19Z-pentaenoic acid (13R-HpDPA); 13R-HpDPA or its reduced alcohol form 13R-hydroxy-docosa-7Z,10Z,14E,16Z,19Z-pentaenoic acid (13R-HDPA) is then donated to neutrophils during neutrophil-endothelial cell interactions, whereby it is first converted to 7-hydro(peroxy)-13R-hydroxy-docosa-8,10Z,14E,16Z,19Z-pentaenoic acid [38]. This molecule can then undergo a second lipoxygenation reaction to yield 7,13R,20-trihydroxy-docosa-8E,10Z,14E,16Z,18E-pentaenoic acid that was coined as RvT1. The hydroperoxide can also, in a lipoxygenase-dependent manner, undergo an epoxidation reaction to yield the allylic epoxide 7,8-11- epoxy-13R-hydroxy-docosa-9,11,14E,16Z,19Z-pentaenoic acid. This is then enzymatically hydrolysed to 7,12,13R-trihydroxy-docosa-8Z,10E,14E,16Z,19Z-pentaenoic acid and 7,8,13R-trihydroxy-docosa-9E,11E,14E,16Z,19Z-pentaenoic acid, coined RvT2 and RvT3, respectively. Finally, 7-hydro(peroxy)-13R-hydroxy-docosa-8,10Z,14E,16Z,19Z-pentaenoic acid is also reduced to 7,13R-dihydroxy-docosa-8,10Z,14E,16Z,19Z-pentaenoic acid that is coined RvT4 (Figure 3).

RvTs are produced in humans during exercise, that is now seen as a self-resolving inflammatory state, marked by an increase in neutrophil-endothelial interactions with upregulation of plasma RvT [38]. These mediators are also produced during infection when RvT levels are upregulated in plasma from septic patients when compared to healthy volunteers.

RvTs are protective in mice during acute inflammation where a mixture of RvT1, RvT2, RvT3 and RvT4 immediately before or 2 hours after intraperitoneal *Escherichia coli* inoculation resulted in host-protection, increasing survival by >60%. Indeed, RvT limited neutrophil recruitment to the site of inflammation, increased phagocytosis and intracellular ROS levels and upregulated macrophage efferocytosis. Of note, the protective actions of RvTs were found to result from the reprogramming of the innate host response since RvT did not display direct bactericidal actions at biologically-relevant concentrations. Additionally, RvT reduced monocyte and macrophage expression of inflammasome components, decreasing caspase-1 and IL-1β expression and lactate dehydrogenase activity, a marker of pyroptosis. RvT also reduced peripheral blood platelet-leukocyte aggregates, an observation associated with reduced systemic inflammation. Exudate macrophage efferocytosis was also increased and a significant reduction in local and systemic eicosanoid levels was found in mice given RvT [38].

Using a total organic synthetic approach, we recently established the complete stereochemistry and biosynthetic role of 13R-HDPA in the RvT pathway [39]. Chirally pure precursors were used in conjunction with stereoselective reactions that installed the configuration at the carbon 13 atom as R and formed geometrically pure double-bond moieties. Synthetic 13R-HDPA was matched with biogenic 13-HDPA, obtained by incubating n-3 DPA with human recombinant COX-2. Structural evaluation of synthetic 13R-HDPA and biogenic 13-HDPA was carried out using liquid chromatography tandem mass spectrometry (LC-MS/MS) to attain MRM chromatograms to match retention times and MS-MS spectra to identify matching daughter ions. Chiral LC-MS/MS was used to confirm the stereochemistry around carbon 13, and UV-Vis spectrophotometry was used to match the double bond conjugation system. Incubation of the synthetic material with human leukocytes demonstrated that this was rapidly converted to all four RvTs [39]. These findings confirmed that the stereochemistry around the hydroxyl group on carbon 13 was in the R orientation and the double bond geometry around the conjugated double bond system was found to be in *E, Z* with the complete stereochemistry

established as 13(R)-hydroxy-7Z,10Z,13R,14E,16Z,19Z-docosapentaenoic acid as well as the role of this intermediate in the RvT biosynthetic pathway.

RvT mediate the biological actions of statins in infectious-inflammation and inflammatory arthritis

SPMs are implicated in mediating the protective actions of a number of clinically relevant therapeutics including aspirin and statins, whereby aspirin initiates the biosynthesis of epimeric forms of SPMs [40], while lovastatin, for example, upregulates the biosynthesis of 15-epi-LXA_4 [41]. In addition, atorvastatin was recently found to increase RvT formation during human neutrophil-endothelial cell interactions, as well as in mice during infections. This increase in RvT production resulted from the S-nitrosylation of COX-2 leading to increased 13R-HDPA levels, suggesting that the S-nitrosylation of COX-2 increased catalytic activity of the enzyme. This finding was in concordance with the S-nitrosylation of COX-2 cysteine residues in the presence of atorvastatin [38]. Inhibition of inducible nitric oxide synthase (iNOS) by L-NG-nitroarginine (L-NAME) reduced the atorvastatin-mediated increases in peritoneal RvT levels after *E. coli* inoculation. A similar reduction was observed when mice were given celecoxib, a COX-2 specific inhibitor. This highlights the complex regulatory axis of COX-2, as post-translational modification of the enzyme may yield mediators with distinct biological activities. In this context, aspirin acetylates COX-2 serine residues and inhibits the production of eicosanoids such as PGE_2, PGD_2 and TxB_2, and upregulates the production of AT-LXA_4.

This mechanism was recently also found to be protective in arthritic inflammation where administration of atorvastatin upregulated RvT concentrations during inflammatory arthritis in both peripheral blood and joints [42]. Of note, this protective mechanism was not unique to atorvastatin and was shared with pravastatin. Atorvastatin administration during ongoing arthritis led to a 43% increase in total RvT amounts in arthritic paws compared to vehicle-treated mice. Pravastatin also increased paw RvT by ~20% with increases in RvT1 and RvT2.

These increases in joint RvT concentrations were also linked with decreases in tissue prostanoids and LTB_4 concentrations. Prostaglandins were reduced by 20-40% by all statins tested when compared to vehicle. Exceptionally, $PGF_{2\alpha}$ was reduced by ~75% in mice given pravastatin. LTB_4 concentrations were reduced ~50% by atorvastatin and ~15% by pravastatin. Additionally, TxB_2 was reduced 20-50% both statins [42]. The upregulation in RvT concentrations were linked with a reduction in disease severity where in mice administered atorvastatin, disease progression was dampened at day 4 post disease initiation, with disease scores reaching a maximum of 9.1 ± 1.2 at day 5 which was sustained until day 7. When mice were administered pravastatin, disease activity at day 5 was lower compared with mice administered vehicle, with a reduction in disease activity maintained until day 7 measured both as reduction in of clinical score and edema. In addition, both statins also lead to a reduction in joint damage at a histological level.

In inflammatory arthritis atorvastatin and pravastatin administration also regulates both circulating and tissue resident leukocyte responses. In non-classical monocytes, atorvastatin reduced the expression of CD11b by ~18% and platelet-monocyte aggregation (measured by a decrease in CD62P) was reduced by ~24% compared to mice given vehicle [42]. Pravastatin significantly reduced platelet-monocyte aggregation by ~35%, and decreased CD11b expression by ~10%. In classical monocytes, expression of CD11b and CD62P were significantly reduced by ~42% and ~34% respectively in mice given atorvastatin compared to mice given vehicle. In mice given pravastatin, CD11b expression was decreased by ~40% and platelet-monocyte aggregation reduced by ~35%. Compared to vehicle, neutrophil activation markers were significantly reduced by atorvastatin and pravastatin, reducing CD11b expression by ~30% and platelet-neutrophil aggregation by ~24%. Of note, administration of celecoxib that inhibits the upregulation of RvT by pravastatin and atorvastatin reverses the protective actions of these statins on both disease severity and leukocyte responses [42].

Conclusion

The identification of n-3 DPA as a substrate to novel, structurally distinct, mediators that display potent host protective activities demonstrates that complex mediator networks become activated during acute inflammation to ensure tissue homeostasis. This is further underscored by the observation of a selective regulation of distinct lipid mediator pathways in a tissue and cell specific manner. In addition, mounting evidence suggests that some of the beneficial actions of a number of widely used drugs, including statins, is mediated via the regulation of these protective pathways. Given the potent actions of n-3 DPA-derived SPMs in regulating systemic and peripheral inflammatory responses, utilizing analogues and mimetics may be useful therapeutics in the prevention and treatment of chronic inflammatory diseases. In addition, strategies to boost their endogenous production potentially via supplementation with n-3 DPA may also be useful in controlling inflammation. While the clinical evidence for this approach is currently limited, recent studies in healthy volunteers provide evidence for the utility of this approach, whereby administering of n-3 DPA was found to upregulate peripheral blood concentrations of $RvD5_{n\text{-}3\ DPA\ [43]}$. Future studies will need to determine which patient populations will be responsive to this approach and which supplement forms will be effective in regulating n-3 DPA-derived SPM concentrations. In this context, changes in their tissue concentrations with both disease and treatment suggest that these pathways may also be useful as biomarkers in both patient stratification and measuring the effectiveness of treatment efficacy.

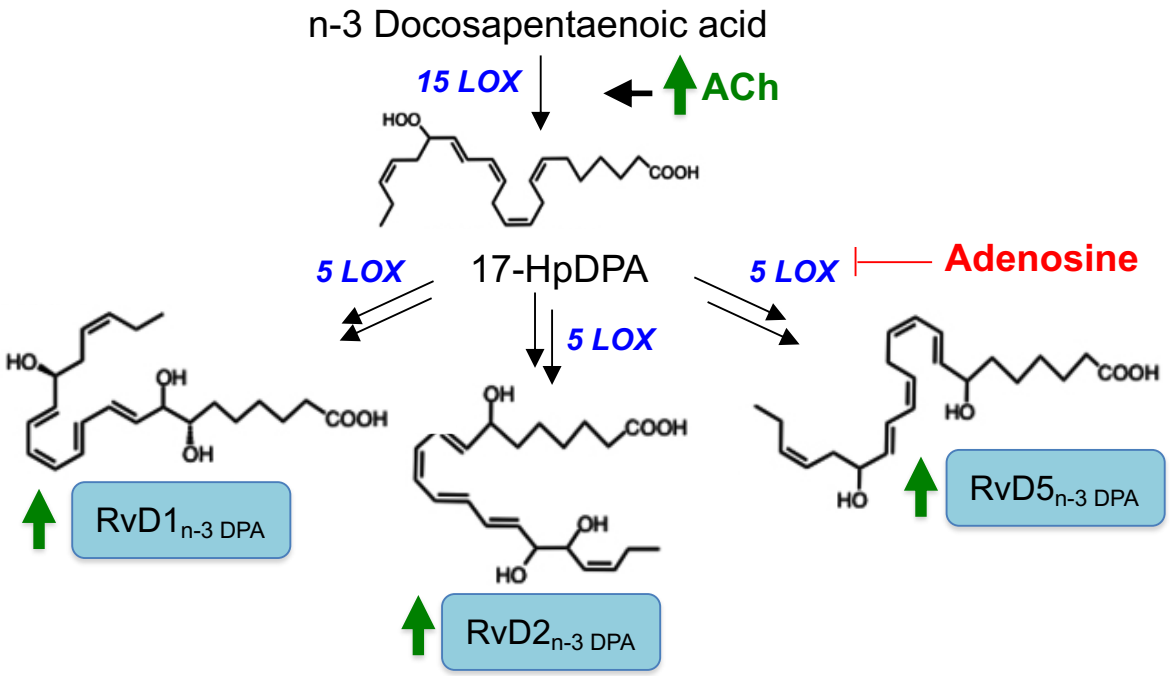
References

1. Malagoli D The evolution of the immune system : conservation and diversification.
2. Majno G (1991) The ancient riddle of sigma eta psi iota sigma (sepsis). J Infect Dis 163 (5):937-945
3. Dalli J, Serhan CN (2018) Identification and structure elucidation of the pro-resolving mediators provides novel leads for resolution pharmacology. Br J Pharmacol. doi:10.1111/bph.14336
4. de Gaetano M, McEvoy C, Andrews D, Cacace A, Hunter J, Brennan E, Godson C (2018) Specialized Pro-resolving Lipid Mediators: Modulation of Diabetes-Associated Cardio-, Reno-, and Retino-Vascular Complications. Front Pharmacol 9:1488. doi:10.3389/fphar.2018.01488
5. Perretti M, Norling LV (2017) Actions of SPM in regulating host responses in arthritis. Mol Aspects Med 58:57-64. doi:10.1016/j.mam.2017.04.005
6. Quiros M, Nusrat A (2019) Saving Problematic Mucosae: SPMs in Intestinal Mucosal Inflammation and Repair. Trends Mol Med. doi:10.1016/j.molmed.2018.12.004
7. Serhan CN, Levy BD (2018) Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest 128 (7):2657-2669. doi:10.1172/JCI97943
8. Serhan CN (2017) Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. Mol Aspects Med 58:1-11. doi:10.1016/j.mam.2017.03.001
9. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 192 (8):1197-1204
10. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med 196 (8):1025-1037
11. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M (2009) Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. J Exp Med 206 (1):15-23. doi:10.1084/jem.20081880
12. Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN (2008) Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. Blood 112 (3):848-855. doi:10.1182/blood-2007-11-122598
13. Morita M, Kuba K, Ichikawa A, Nakayama M, Katahira J, Iwamoto R, Watanebe T, Sakabe S, Daidoji T, Nakamura S, Kadowaki A, Ohto T, Nakanishi H, Taguchi R, Nakaya T, Murakami M, Yoneda Y, Arai H, Kawaoka Y, Penninger JM, Arita M, Imai Y (2013) The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell 153 (1):112-125. doi:10.1016/j.cell.2013.02.027
14. Dalli J, Vlasakov I, Riley IR, Rodriguez AR, Spur BW, Petasis NA, Chiang N, Serhan CN (2016) Maresin conjugates in tissue regeneration biosynthesis enzymes in human macrophages. Proc Natl Acad Sci U S A 113 (43):12232-12237. doi:10.1073/pnas.1607003113
15. Chiang N, Serhan CN (2017) Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med 58:114-129. doi:10.1016/j.mam.2017.03.005

16. Colas RA, Dalli J, Chiang N, Vlasakov I, Sanger JM, Riley IR, Serhan CN (2016) Identification and Actions of the Maresin 1 Metabolome in Infectious Inflammation. J Immunol 197 (11):4444-4452. doi:10.4049/jimmunol.1600837
17. Calder PC (2011) Fatty acids and inflammation: the cutting edge between food and pharma. Eur J Pharmacol 668 Suppl 1:S50-58. doi:10.1016/j.ejphar.2011.05.085
18. De Caterina R (2011) n-3 fatty acids in cardiovascular disease. N Engl J Med 364 (25):2439-2450. doi:10.1056/NEJMra1008153
19. Crawford MA, Broadhurst CL, Guest M, Nagar A, Wang Y, Ghebremeskel K, Schmidt WF (2013) A quantum theory for the irreplaceable role of docosahexaenoic acid in neural cell signalling throughout evolution. Prostaglandins Leukot Essent Fatty Acids 88 (1):5-13. doi:10.1016/j.plefa.2012.08.005
20. Dalli J, Colas RA, Serhan CN (2013) Novel n-3 immunoresolvents: structures and actions. Sci Rep 3:1940. doi:10.1038/srep01940
21. Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, Nettleton JA, King IB, Weng LC, Bhattacharya S, Bandinelli S, Bis JC, Rich SS, Jacobs DR, Jr., Cherubini A, McKnight B, Liang S, Gu X, Rice K, Laurie CC, Lumley T, Browning BL, Psaty BM, Chen YD, Friedlander Y, Djousse L, Wu JH, Siscovick DS, Uitterlinden AG, Arnett DK, Ferrucci L, Fornage M, Tsai MY, Mozaffarian D, Steffen LM (2011) Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. PLoS Genet 7 (7):e1002193. doi:10.1371/journal.pgen.1002193
22. Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. Nat Immunol 6 (12):1191-1197. doi:10.1038/ni1276
23. Aursnes M, Tungen JE, Vik A, Colas R, Cheng CY, Dalli J, Serhan CN, Hansen TV (2014) Total synthesis of the lipid mediator PD1n-3 DPA: configurational assignments and anti-inflammatory and pro-resolving actions. J Nat Prod 77 (4):910-916. doi:10.1021/np4009865
24. Gobbetti T, Dalli J, Colas RA, Federici Canova D, Aursnes M, Bonnet D, Alric L, Vergnolle N, Deraison C, Hansen TV, Serhan CN, Perretti M (2017) Protectin D1n-3 DPA and resolvin D5n-3 DPA are effectors of intestinal protection. Proc Natl Acad Sci U S A 114 (15):3963-3968. doi:10.1073/pnas.1617290114
25. Colas RA, Souza PR, Walker ME, Burton M, Zaslona Z, Curtis AM, Marques RM, Dalli J (2018) Impaired Production and Diurnal Regulation of Vascular RvDn-3 DPA Increase Systemic Inflammation and Cardiovascular Disease. Circ Res 122 (6):855-863. doi:10.1161/CIRCRESAHA.117.312472
26. Ingle KA, Kain V, Goel M, Prabhu SD, Young ME, Halade GV (2015) Cardiomyocyte-specific Bmal1 deletion in mice triggers diastolic dysfunction, extracellular matrix response, and impaired resolution of inflammation. Am J Physiol Heart Circ Physiol 309 (11):H1827-1836. doi:10.1152/ajpheart.00608.2015
27. McAlpine CS, Swirski FK (2016) Circadian Influence on Metabolism and Inflammation in Atherosclerosis. Circ Res 119 (1):131-141. doi:10.1161/CIRCRESAHA.116.308034
28. Puttonen S, Oksanen T, Vahtera J, Pentti J, Virtanen M, Salo P, Kivimaki M (2010) Is shift work a risk factor for rheumatoid arthritis? The Finnish Public Sector study. Ann Rheum Dis 69 (4):779-780. doi:10.1136/ard.2008.099184
29. Krump E, Picard S, Mancini J, Borgeat P (1997) Suppression of leukotriene B4 biosynthesis by endogenous adenosine in ligand-activated human neutrophils. J Exp Med 186 (8):1401-1406

30. Viola JR, Lemnitzer P, Jansen Y, Csaba G, Winter C, Neideck C, Silvestre-Roig C, Dittmar G, Doring Y, Drechsler M, Weber C, Zimmer R, Cenac N, Soehnlein O (2016) Resolving Lipid Mediators Maresin 1 and Resolvin D2 Prevent Atheroprogression in Mice. Circ Res 119 (9):1030-1038. doi:10.1161/CIRCRESAHA.116.309492
31. Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B, Connolly ES, Solomon R, Jones DM, Heyer EJ, Spite M, Tabas I (2016) An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun 7:12859. doi:10.1038/ncomms12859
32. Buckley CD, McGettrick HM (2018) Leukocyte trafficking between stromal compartments: lessons from rheumatoid arthritis. Nat Rev Rheumatol 14 (8):476-487. doi:10.1038/s41584-018-0042-4
33. Tabas I, Lichtman AH (2017) Monocyte-Macrophages and T Cells in Atherosclerosis. Immunity 47 (4):621-634. doi:10.1016/j.immuni.2017.09.008
34. Pistorius K, Souza PR, De Matteis R, Austin-Williams S, Primdahl KG, Vik A, Mazzacuva F, Colas RA, Marques RM, Hansen TV, Dalli J (2018) PDn-3 DPA Pathway Regulates Human Monocyte Differentiation and Macrophage Function. Cell Chem Biol 25 (6):749-760 e749. doi:10.1016/j.chembiol.2018.04.017
35. Primdahl KG, Tungen JE, De Souza PRS, Colas RA, Dalli J, Hansen TV, Vik A (2017) Stereocontrolled synthesis and investigation of the biosynthetic transformations of 16(S),17(S)-epoxy-PDn-3 DPA. Org Biomol Chem 15 (40):8606-8613. doi:10.1039/c7ob02113e
36. Chiang N, Serhan CN (2017) Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med. doi:10.1016/j.mam.2017.03.005
37. Dalli J, Serhan C (2016) Macrophage Proresolving Mediators-the When and Where. Microbiol Spectr 4 (3). doi:10.1128/microbiolspec.MCHD-0001-2014
38. Dalli J, Chiang N, Serhan CN (2015) Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. Nat Med 21 (9):1071-1075. doi:10.1038/nm.3911
39. Primdahl KG, Aursnes M, Walker ME, Colas RA, Serhan CN, Dalli J, Hansen TV, Vik A (2016) Synthesis of 13(R)-Hydroxy-7Z,10Z,13R,14E,16Z,19Z Docosapentaenoic Acid (13R-HDPA) and Its Biosynthetic Conversion to the 13-Series Resolvins. J Nat Prod 79 (10):2693-2702. doi:10.1021/acs.jnatprod.6b00634
40. Claria J, Serhan CN (1995) Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci U S A 92 (21):9475-9479
41. Planaguma A, Pfeffer MA, Rubin G, Croze R, Uddin M, Serhan CN, Levy BD (2010) Lovastatin decreases acute mucosal inflammation via 15-epi-lipoxin A4. Mucosal Immunol 3 (3):270-279. doi:10.1038/mi.2009.141
42. Walker ME, Souza PR, Colas RA, Dalli J (2017) 13-Series resolvins mediate the leukocyte-platelet actions of atorvastatin and pravastatin in inflammatory arthritis. FASEB J 31 (8):3636-3648. doi:10.1096/fj.201700268
43. Markworth JF, Kaur G, Miller EG, Larsen AE, Sinclair AJ, Maddipati KR, Cameron-Smith D (2016) Divergent shifts in lipid mediator profile following supplementation with n-3 docosapentaenoic acid and eicosapentaenoic acid. FASEB J 30 (11):3714-3725. doi:10.1096/fj.201600360R

Figure Legends



Counter regulate physiological peripheral blood platelet, neutrophil and monocyte activation

Loss of $RvD_{n\text{-}3\ DPA}$ production leads to increased peripheral blood leukocyte and platelet activation and is linked with CVD

Figure 1: Diurnal changes in $RvD_{n\text{-}3\ DPA}$ regulates peripheral blood leukocyte and platelet activation protecting from cardiovascular disease. In peripheral blood diurnal changes in acetylcholine (ACh) upregulates 15-lipoxygenase (ALOX15) activity promoting $RvD_{n\text{-}3\ DPA}$ biosynthesis that limit the physiological activation of monocytes, neutrophils and platelets during the early morning hours. Increases in circulating adenosine concentrations in patients with cardiovascular disease inhibit ALOX5 activity disrupting the diurnal changes in plasma $RvD_{n\text{-}3\ DPA}$ and increasing peripheral blood leukocyte and platelet activation.

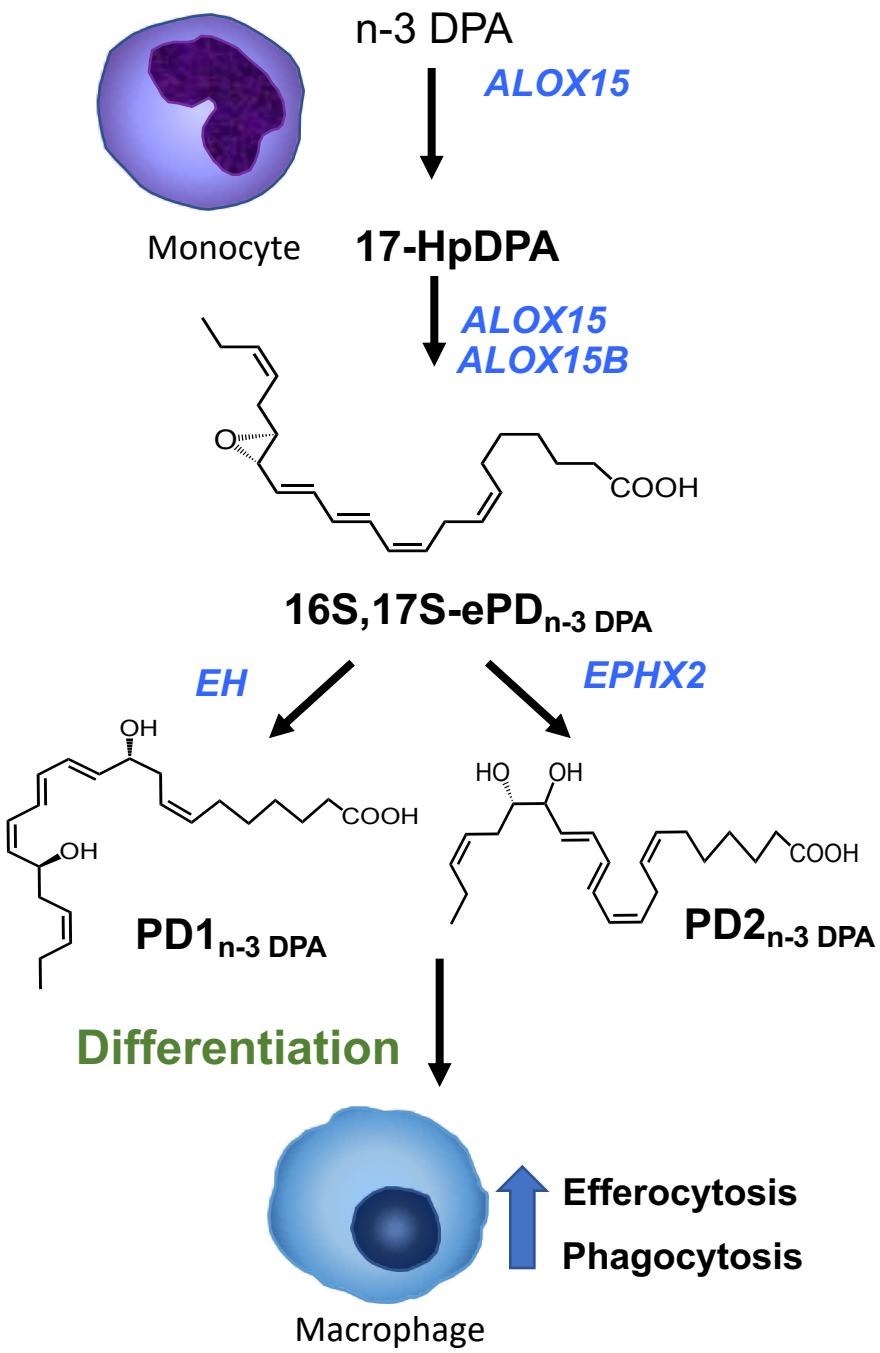
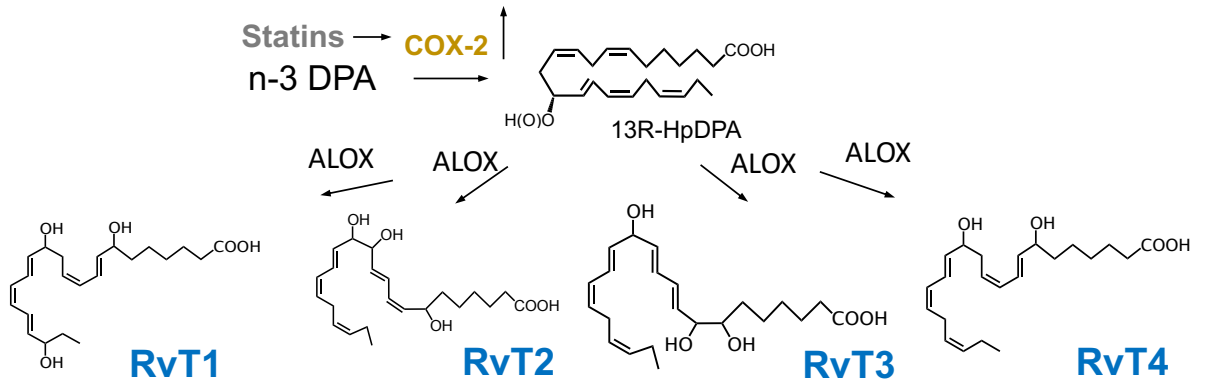


Figure 2: $PD_{n\text{-}3\ DPA}$ biosynthetic pathway and its regulation of monocyte-derived macrophage phenotype and function. In the $PD_{n\text{-}3\ DPA}$ pathway n-3 DPA is converted to 17-HpDHA and then to 16S, 17S-epoxy-$PD_{n\text{-}3\ DPA}$ by either ALOX15 or ALOX15B. This is then hydrolyzed by epoxide hydrolase activity to $PD1_{n\text{-}3\ DPA}$ and $PD2_{n\text{-}3\ DPA}$. This pathway regulates macrophage phenotype during monocyte-to-macrophage differentiation.



Bacterial Infections	Inflammatory Arthritis
• Limit neutrophil activation and recruitment • Increase vascular PGI_2 • Downregulate inflammasome activation • Counter-regulate the production of inflammatory mediators • Enhance neutrophil and macrophage phagocytosis of bacteria • Upregulate efferocytosis • Increase survival from lethal infections	• Limit neutrophil activation and recruitment to the joint • Regulate joint monocyte and macrophage phenotype • Reduce peripheral blood monocyte, neutrophil and platelet activation • Counter-regulate the production of inflammatory mediators • Reduce joint damage

Figure 3: RvT biosynthesis and actions in mediating the protective actions of statins in infections and inflammatory arthritis. Production of RvT is initiated via the conversion of n-3 DPA by the endothelial COX-2 expression yielding 13-HpDHA, that can then be converted to 13-HDHA and donated to neutrophils where via ALOX activity this is converted to RvT1-4. Statin mediated S-nitrosylation of COX-2 upregulates 13-HDPA production contributing to the upregulation of RvTs.