Eicosapentaenoic and docosahexaenoic acid derived specialised pro-resolving mediators: concentrations in humans and the effects of age, sex, disease and increased omega-3 fatty acid intake

Philip C. Calder[1,2]

[1]School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, United Kingdom;

[2]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, United Kingdom

Author's details: School of Human Development and Health, Faculty of Medicine, University of Southampton, IDS Building, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, United Kingdom.

Email: pcc@soton.ac.uk

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Abbreviations used: AT, aspirin-triggered; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GPR, G protein coupled receptor; IL, interleukin; LOX, lipoxygenase; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LT, leukotriene; MaR, maresin; PD1, protectin D1; PDX, protectin X; PG, prostaglandin; PUFA, polyunsaturated fatty acid; Rv, resolvin; SPM, specialised pro-resolving lipid mediator.

Abstract

Although inflammation has a physiological role, unrestrained inflammation can be detrimental, causing tissue damage and disease. Under normal circumstances inflammation is self-limiting with induction of active resolution processes. Central to these is the generation of specialised pro-resolving lipid mediators (SPMs) from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These include resolvins, protectins and maresins whose activities have been well described in cell and animal models. A number of SPMs have been reported in plasma or serum in infants, children, healthy adults and individuals with various diseases, as well as in human sputum, saliva, tears, breast milk, urine, synovial fluid and cerebrospinal fluid and in human adipose tissue, skeletal muscle, hippocampus, skin, placenta, lymphoid tissues and atherosclerotic plaques. Differences in SPM concentrations have been reported between health and disease, as would be expected. However, sometimes SPM concentrations are lower in disease and sometimes they are higher. Human studies report that plasma or serum concentrations of some SPMs can be increased by increasing intake of EPA and DHA. However, the relationship of specific intakes of EPA and DHA to enhancement in the appearance of specific SPMs is not clear and needs a more thorough investigation. This is important because of the potential for EPA and DHA to be used more effectively in prevention and treatment of inflammatory conditions. If generation of SPMs represents an important mechanism of action of EPA and DHA, then more needs to be known about the most effective strategies by which EPA and DHA can increase SPM concentrations.

1. Introduction and scope

Inflammation is a normal part of the immune response. It acts to help in protection against pathogens and is involved in the response to injury and in wound healing [1,2]. The inflammatory response involves many cell types and a plethora of chemical mediators [1,2]. The latter include lipids, peptides, reactive oxygen species, amino acid derivatives and enzymes. The exact mix of chemicals produced depends upon the types of cell involved, the nature of the inflammatory stimulus, where the inflammation is occurring, and the stage during the inflammatory response [1,2]. Lipids including prostaglandins (PGs) and leukotrienes (LTs) derived from the omega-6 (n-6) fatty acid arachidonic acid, endocannabinoids and platelet activating factor are well recognised mediators of inflammation and are all generated from cell membrane phospholipids, or their metabolic products. Because the inflammatory response is designed to be damaging to pathogens, it can also damage host tissues [1,2]. Therefore, it is important that inflammation is self-limiting and resolves, which can occur rapidly in some settings. The resolution of inflammation occurs because various inhibitory mechanisms are activated as inflammation runs its course [3,4]. These mechanisms include the shedding of receptors for pro-inflammatory cytokines and the increased generation of anti-inflammatory cytokines [3]. Another central mechanism involved is the generation of specialised pro-resolving lipid mediators (SPMs) which act to inhibit pro-inflammatory signalling [4]. Amongst the most important SPMs are lipoxin A_4 generated from arachidonic acid [5-7] and a series of mediators generated from the omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic, docosapentaenoic and docosahexaenoic acid [8-11]. Failure to resolve inflammation can result in excessive, inappropriate or on-going inflammation that can cause irreparable damage to host tissues leading to pathology and disease (Figure 1). N-3 PUFAs are recognised to have anti-inflammatory actions, acting to decrease activation of inflammatory cells and the production of classic inflammatory mediators like PGs and LTs from arachidonic acid and a range of pro-inflammatory cytokines and chemokines [12-14]. The recognition that n-3 PUFAs are substrates for the generation of SPMs suggests that the availability of these fatty acids in the bloodstream and in cell membranes is central to inflammation resolution and to protection of the host against the deleterious consequences of overzealous inflammation (Figure 2). Most research on the biology of SPMs has rightly focussed on their appearance in various controlled cellular and animal models of inflammation and its resolution and in the use of individual SPMs in treating inflammation in those models and the mechanisms involved; in these regards tremendous progress has been made [3,4,8-11,15-19]. There has been less attention paid to the appearance and roles of SPMs in humans, to sources of any variation that may occur in their concentrations,

and to the role of n-3 PUFAs as endogenous substrates in promoting their synthesis. This review will summarise the literature that currently exists on the concentrations of SPMs derived from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans; on whether there is evidence that these concentrations differ according to age, sex and disease; and on whether these concentrations can be increased through oral provision of EPA and DHA.

2. EPA and DHA as substrates for synthesis of SPMs

SPMs include resolvins (Rvs), protectins (also known as neuroprotectins) and maresins (MaRs). E- and D-series Rvs are produced from EPA and DHA, respectively, while protectins and MaRs are produced from DHA. SPMs are synthesised using cyclooxygenase (COX) and lipoxygenase (LOX) enzymes often working within the same pathway, outlines of which are provided in Figures 3 and 4. Synthesis of many SPMs is influenced by aspirin and in the presence and absence of aspirin different epimers of these SPMs, which can have different biological activity or potency, are produced [4,8-10,16-19]. Those epimers favoured by the presence of aspirin have been referred to as aspirin-triggered (AT). In recent years cysteinyl conjugates of several SPMs have been identified [17,20]; such conjugated MaRs have been termed maresin conjugates in tissue regeneration.

SPMs act via G protein coupled receptors (GPRs) [18]; these receptors are typically able to interact with more than one SPM and conversely some SPMs are able to interact with several receptors. For example, RvD1, AT-RvD1 and RvD3 interact with lipoxin A_4 receptor/N-formyl peptide receptor 2 (ALX/FPR2) and with GPR32 (also known as resolvin D1 receptor (DRV1)); RvD5 interacts with GPR32; RvD2 interacts with GPR18 (also known as resolvin D2 receptor (DRV2)); and RvE1 and E2 interact with chemokine like receptor 1 (also known as chemerin receptor 23 (ChemR23) and resolvin E receptor (ERV)). MaR1 interacts with leucine-rich repeat-containing G-protein coupled receptor 6 (LGR6) and also with the nuclear receptor RAR-related orphan receptor alpha (RORα; also known as nuclear receptor subfamily 1, group F, member 1 (NR1F1)). SPMs can also antagonise some pro-inflammatory receptors. For example, RvE1 and MaR1 are competitive antagonists of the LTB_4 receptor (BLT1).

The biological effects of the various SPMs have been examined extensively in cell culture and animal models of inflammation and are reviewed and discussed elsewhere [8-11;15-19]. These model systems have demonstrated that all SPMs tested to date have anti-inflammatory and inflammation resolving actions. As examples, RvE1, RvD1 and protectin D1 (PD1) all inhibit transendothelial migration of neutrophils, so preventing the infiltration of neutrophils into sites

of inflammation; RvD1 inhibits interleukin (IL)-1β production; and PD1 inhibits tumour necrosis factor and IL-1β production [8-10]. Various SPMs reduce inflammation and are protective in many experimental animal models of inflammatory disease including arthritis [21,22], colitis [23] and asthma [24-27] and in other states of inflammation including sepsis [28,29] and acute lung injury [30-32].

3. EPA- and DHA-derived SPMs in different body pools in humans

3.1 Blood plasma and serum

Table 1 summarises a number of human studies reporting the concentrations of EPA- and DHA-derived SPMs in plasma or serum [33-72]. Most studies have used liquid chromatography with tandem mass spectrometry (LC-MS/MS) for analysis although some have used commercial immunoassays. These different analytical approaches seem to produce different concentrations of SPMs (Table 1). Using LC-MS/MS, a number of SPMs are commonly reported in plasma and serum of healthy adults including 18R-RvE1, 18R-RvE2, 18R-RvE3, RvD1, RvD2, RvD3, RvD4, RvD5, AT-RvD1, PD1 and MaR1 (Table 1). Others, including AT-PD1, PDX and MaR2 have also been reported in some studies and 18S-RvE3 is also sometimes reported (Table 1). Low concentrations of SPMs can be an analytical challenge and SPMs reported in some studies are described as being not detected in others (Table 1). This may reflect low concentrations that fall below the limits of detection in some laboratories, which will be influenced by sample storage and processing and analytical instrumentation. Furthermore, of course, not all studies have attempted to identify all SPMs that might or might not be detected. Therefore, the full range of SPMs circulating in the human bloodstream at detectable concentrations is not currently known. There are large between study and within study variations in the concentrations of SPMs reported, even when very similar, or identical, analytical approaches are used (Table 1). Figure 5 depicts the range of average concentrations of 11 SPMs that have been reported in plasma or serum from healthy humans using LC-MS/MS in different studies. The extent to which these differences reflect meaningful biological variation is not clear.

While most studies have reported SPM concentrations in plasma, some report serum concentrations (Table 1). A study compared concentrations of two SPMs in plasma and serum prepared from the same blood sample [58]. Concentrations of RvD1 and PD1 were lower by 22% and 32%, respectively, in serum than in plasma from healthy participants, but both were

higher by about 100% in serum from patients with psoriasis than in plasma. It is difficult to interpret these findings.

SPMs have been reported in umbilical cord plasma and in plasma of infants and children tracked over time [50]. These concentrations are generally similar to those reported for healthy adults by the same laboratory [38].

3.2 Other body fluids: Sputum, saliva, tears, breast milk, urine, synovial fluid and cerebrospinal fluid

Selected studies reporting SPMs in various body fluids in humans, other than blood, are summarised in Table 2 [36,44,64,74-87]. There are a limited number of studies in any one fluid. Sputum RvE1 and RvD1 have been reported to be positively associated with lung function in patients with cystic fibrosis [74.75]. Studies using LC-MS/MS and ELISA reveal several SPMs in saliva but report very different concentrations [36,76,77]. Where individuals with periodontitis have been compared with healthy controls, SPM concentrations in saliva, where detectable, seem little different [36,77]. A higher salivary ratio of RvD1 to LTB_4 was associated with lower carotid intima media thickness in older individuals with co-morbidities [76]. Most SPMs have not been detected in tears [78,79], but some SPMs were detected in tears from some males [79]. A number of SPMs have been reported in human breast milk [44,80,81]. Concentrations of both 18R-RvE1 and RvD1 were similar in milk provided at different times across the first month of lactation [80]. A higher concentration of 18R-RvE1 was seen if breast milk came from women with mastitis [81]. Nevertheless, the number of breast milk samples analysed in these studies was quite low. RvD5 and MaR1 have been reported in synovial fluid from patients with arthritis [83], but how these compare with the non-arthritic condition is not known. Some SPMs are present in urine [64,82], but the effects of smoking tobacco or using e-cigarettes on urinary SPMs are unclear [64,82]. Some SPMs have been reported in cerebrospinal fluid in different conditions using LC-MS/MS [84,87] or ELISA [85,86]. RvD1 concentration was much lower in cerebrospinal fluid in patients with neuromyelitis optica spectrum disorder or multiple sclerosis than in healthy controls [86].

3.3 Adipose tissue, skeletal muscle, brain, skin, placenta, lymph tissues and atherosclerotic plaque

Selected studies reporting SPMs in various tissues in humans are summarised in Table 3 [37,58,66,85,88-91]. There are a limited number of studies in any one tissue. RvD1, RvD2 and PD1 have all been reported in human subcutaneous adipose tissue, with higher concentrations of RvD1 and lower concentrations of PD1 in tissue from patients with peripheral artery disease than from controls [88]. Likewise, RvD2 was detected in skeletal muscle from patients with peripheral artery disease but not in muscle from healthy controls [89]. MaR1 was detected in the hippocampus of deceased heathy individuals aged about 80 years, but not in those with Alzheimer's Disease [85]. Two studies on skin concentrations of SPMs report very different concentrations and a lack of consistency in patterns seen with skin disease [58,66]. Skin affected by atopic dermatitis how low concentrations of RvD1 which were similar to concentrations seen in skin of heathy individuals but much lower than seen in the non-affected skin of the same patients [66]. Affected skin of patients with atopic dermatitis had lower concentrations of RvD2 than seen in both non-affected skin and skin of heathy subjects [58]. Skin PD1 and MaR1 were little different among affected and non affected skin of patients and skin of healthy controls [66]. RvD5 was easily detectable in lesional skin of patients with psoriasis but was not detected in non-lesional skin of those patients or in skin of healthy controls [66]. PD1 was higher in non-lesional skin than lesional skin of patients with psoriasis but was not detected in skin of heathy controls [66]. Some SPMs have been reported in human placenta [90] and in post-mortem spleen and lymph nodes [37]. Most SPMs were not detected in advanced atherosclerotic plaques, but RvD1 was much higher in stable than unstable plaques although PD1 was not different [91].

4. Effects of sex on EPA and DHA-derived SPMs

Females have higher capacity than males for endogenous synthesis of EPA and DHA from alpha-linolenic acid [92] and a meta-analysis of 51 studies revealed that females have higher plasma concentrations of DHA than males [93]. The higher availability of DHA in females may mean greater capacity to produce D-series Rvs, protectins and maresins than in males. Many studies have reported data on SPM concentrations for mixed groups of males and females (Tables 1, 2 and 3) but there are few explorations of sex differences in these. Barden et al. [41] identified that the plasma concentrations of the SPM precursors 18-hydroxy-EPA, 14-hydroxy DHA and 17 hydroxy-DHA were higher in females than males, but that there were no sex differences in the concentrations of 18R-RvE1, 18R-RvE2, 18R-RvE3, 18S-RvE3, RvD1, RvD2, AT-RvD1, PD1 or MaR1. More recently, Barden et al [60] reported that plasma RvE2,

RvD1 and AT-RvD1 were lower in females compared to males, and that plasma RvD2 was not different between the sexes. Rathod et al. [94] profiled lipid mediators in the fluid of skin blisters 24 hr after their induction by cantharidin. The blisters contained mediators formed from arachidonic acid, EPA and DHA, including RvD1, RvD2, RvD3 and PD1. They identified distinct clusters of lipid mediators in females and males, with some SPM concentrations being numerically higher in females, although only PD1 was significantly different between sexes. The sum of all SPMs was 17.2 pg/50 µl in females and 10.5 pg/50 µl in males. The sum of D-series Rvs was significantly higher in females while pro-inflammatory LTB_4 was significantly lower. In a separate experiment, plasma SPMs were measured in females and males 8 hr following typhoid vaccination [94]. LTB_4 was higher in males and was associated to the male lipid mediator cluster, while RvE1 and RvE3 were associated with the female lipid mediator cluster. However, no EPA- or DHA-derived SPM reported (18R-RvE1, 18R-RvE2, 18R-RvE3, RvD1, EvD2, RvD3, RvD4, RvD5, RvD6, AT-RvD1, AT-RvD3, PD1, AT-PD1) was different between the sexes, although the sum of E-series Rvs was significantly higher in females. Taking the findings of Barden et al. [41,60] and Rathod et al. [94] together suggests that differences in concentrations of individual SPMs in plasma between females and males may be small, although the data of Rathod et al. [94] suggest that the total "resolvome" may be more pro-resolving in females than males.

5. Effects of age on EPA and DHA-derived SPMs

The metabolism of n-3 PUFAs (both EPA and DHA) changes with aging [95,96] and there are reports of altered EPA and DHA status in blood, cells and tissues in older compared to younger adults [97,98,99]. Aging is associated with chronic low-grade inflammation, that predisposes to age-related morbidities [100], suggesting a loss of pro-resolution activity. Despite the many studies reporting data on SPM concentrations in humans (Tables 1, 2 and 3), there are few explorations of age differences in these. Jove et al. [101] conducted non-targeted metabolic analysis of plasma of 146 healthy individuals (68 males and 78 females) aged 39 to 100 yr. Out of 2,678 metabolites identified, 5 were associated with age (all inversely) in both males and females [90]; amongst these metabolites was RvD6. The other four metabolites were a vitamin D_2-related compound, a specific phosphoserine, a specific monoacylglycerol and a specific diacylglycerol

6. EPA and DHA-derived SPMs in human disease

Many diseases are characterised by chronic inflammation [1-3,100]; this may be due, at least in part, to loss to pro-resolution factors. Hence, disease, especially inflammatory disease, might be characterised by lower than usual levels of SPMs. On the other hand, it is possible that on-going inflammation might induce pro-resolving factors to levels that are higher than normal but that they may be ineffective because of an imbalance between pro-inflammatory and pro-resolving mediators (in favour of the former) or because of loss of sensitivity. The study of the concentrations of SPMs in disease states is therefore likely to be informative about the pathophysiology of inflammatory disease and also about strategies to intervene for clinical benefit.

A number of studies report lower concentrations of some SPMs in plasma/serum, in other bodily fluids or in tissues in disease compared to the healthy state or in more severe compared to less severe disease. For example, concentrations of several D-series Rvs were numerically lower in serum of patients with rheumatoid arthritis than in controls, with the difference being significant for RvD3 and almost significant for RvD4 [45]. Likewise, the concentration of RvD1 was significantly lower in plasma of patients with lupus than in healthy controls [57]. Serum RvD1, RvD2 and PD1 concentrations were lower in patients with atopic dermatitis than in healthy controls [66]. Concentrations of RvD1 and RvD2 were lower in affected skin in atopic dermatitis than in non-affected skin [66], while PD1 was lower in lesional than non-lesional skin in psoriasis [58]. MaR1 was found in the hippocampus of controls but not of patients with Alzheimer's Disease [85]. Patients with cystic fibrosis and detectable RvE1 in sputum had better lung function than patients with non-detectable levels [74]. Similarly, the RvD1 to IL-8 ratio was positively related to lung function in patients with cystic fibrosis [75]. There are a series of observations relating to low SPM concentrations and atherosclerotic plaques. A higher salivary ratio of RvD1 to LTB_4 was associated with lower carotid intima media thickness [76], a marker of atherosclerosis. Serum RvD1 was lower by about 50% in patients with unstable plaques compared to those with asymptomatic plaques [53]. Fredman et al. [91] reported that RvD1 was much lower in vulnerable than stable plaques. Finally, plasma concentrations of RvD1 and RvD5 were lower in patients on admission to intensive care than in controls [55]. These observations are all consistent with the idea that many diseases, particularly those with a strong inflammatory component, are associated with a loss of, or at least a diminution in, pro-resolution factors including SPMs. Nevertheless, Barden et al. [41] and Freier et al. [52] reported no differences in plasma and serum concentrations of a number of EPA- and DHA-derived SPMs between patients with metabolic disease and healthy controls.

In contrast the findings described above, there are also several studies reporting higher concentrations of SPMs in disease than in health. For example, serum RvD2 and PD1 concentrations were higher in patients with aggressive periodontitis than in healthy controls [36], while serum RvD1 and PD1 concentrations were higher in patients with psoriasis than in healthy controls, although this difference was not seen in plasma [58]. In contrast to what was seen with PD1, RvD5 concentrations were high in lesional skin in psoriasis but RvD5 was not detected in non-lesional skin or in skin from healthy controls [58]. Plasma RvD1, RvD5 and PD1 concentrations were higher in patients with multiple sclerosis than in healthy controls, with concentrations differing according to disease severity [70]. Plasma 18R-RvE1 was higher in breast milk from women with mastitis than without [81]. Cerebrospinal fluid RvD1concentration was higher in highly active multiple sclerosis than in less active disease, and PD1 was only detectable in some patients with highly active disease [84]. RvD2 was found in skeletal muscle of patients with peripheral artery disease but was not detected in muscle from healthy controls [89]. Plasma lipid mediator profiles were examined in 22 patients with sepsis within 48 hours of admission to the intensive care unit and on days 3 and 7 thereafter [54]. In addition to classic inflammatory lipid mediators like $PGF_{2\alpha}$ and LTB_4, some pro-resolving mediators including 18R-RvE1 and PD1 were higher in plasma of non-survivors than survivors. Furthermore, higher concentrations of inflammation initiating mediators, including $PGF_{2\alpha}$, and of some SPMs were associated with the development of acute respiratory distress syndrome. These findings differ from murine experiments where a range of SPMs protect against sepsis [28,28] and acute lung injury [30-32]. Studies reporting higher levels of SPMs in some diseases perhaps reflect the exaggerated inflammatory response which has induced resolution pathways as part of the normal attempt to restore homeostasis, but which fails perhaps because of an imbalance between the strong ongoing inflammation and an insufficiently strong resolution response. Alternatively, this situation may reflect a loss of responsiveness to pro-resolution signals either because of reduced receptor expression or failure of post-receptor signalling pathways

7. Effects of increased intake of omega-3 fatty acids on SPM concentrations

Since EPA and DHA are precursors for the biosynthesis of SPMs (Figures 3 and 4), it is self evident that the endogenous production of SPMs is dependent, at least to some extent, upon the availability of EPA and DHA. DHA is usually more abundant than EPA in different body pools

in humans, including in blood plasma/serum, leukocytes, erythrocytes, platelets and tissues (see Table 3 of [102]). Increased intake of EPA and DHA results in increased amounts of EPA and DHA in blood lipids, leukocytes, platelets and tissues [103]. The increase in content of EPA and DHA is highly related to the increase in intake (Figures 6 and 7). As discussed in detail elsewhere [13], increased n-3 PUFA content of leukocytes and platelets has been shown to result in reduced capacity to produce eicosanoids from arachidonic acid including PGs and LTs and to enhanced production of some EPA-derived eicosanoids [12-14]. Thus, human studies demonstrate that increased intake of EPA and DHA (usually in combination) is able to modify the production of bioactive lipid mediators involved in inflammatory processes. Hence, it seems reasonable to assume that increased intake of EPA and DHA would result in increased capacity to produce n-3 PUFA-derived SPMs and to result in higher blood (and tissue) concentrations of those SPMs. A number of studies investigating whether increased intake of n-3 PUFAs increases blood concentrations of SPMs have reported increases in the concentrations of the immediate precursors of SPMs such as 18-hydroxy-EPA, 14-hydroxy-DHA and 17-hydroxy-DHA. Analysis of plasma samples from a 12-month long study in healthy participants who consumed increased amounts of EPA and DHA provides significant insight into the effect on concentrations of EPA- and DHA-derived oxylipins [104]. Increases in the plasma concentrations of a number of epoxy, hydroxy and dihydroxy derivatives of both EPA and DHA were reported [104]. These increases were highly correlated with the increase in intake of EPA and DHA, as shown for 18-hydroxy-EPA and 17-hydoxy-DHA in Figures 6 and 7, respectively. Thus, it seems clear that increased intake of EPA and DHA increases the circulating concentrations of the immediate precursors of Rvs, protectins and maresins, presumably reflecting cellular production of these precursor species. Table 4 summarises selected studies that have investigated the effects of increased intake of n-3 PUFAs on SPM concentrations in human body fluids, most often plasma or serum [35,37,38,40-43,46,48,50,59,63,105,106]. These have used a variety of study designs and populations, have used different doses of n-3 PUFAs and been of different durations. Some studies do not provide data for pre-n-3 PUFA supplementation SPM concentrations or concentrations in a comparator control group [105,106] which makes it impossible to fully interpret the findings. Nevertheless, a number of studies report that plasma or serum concentrations of some SPMs are higher after a period of increased intake of EPA and DHA ([35,38,40-43,46,59,63]; Table 4). One study reporting no effect of n-3 PUFAs on plasma SPM concentrations was conducted in infants [50] and it is possible that intakes used were too low to have an effect. In adults, increases in concentrations of 18R-RvE1, 18R-RvE2, 18R-RvE3, RvD1 and RvD2 have been reported several times, but effects on AT-RvD1, other D-series Rvs, PD1

and MaR1 are less consistently seen (Table 4). Figure 8A shows the average % change in concentration reported for five SPMs across several studies, showing the biggest effects are on E-series Rvs. This might reflect the higher EPA than DHA content of most n-3 PUFA preparations used. Figure 8B depicts the reported % change in concentration of RvE1, RvE2 and RvD1 in individual studies in relation to the EPA or DHA intake used in those studies. This figure demonstrates that there may be a threshold intake of n-3 PUFAs that is required before plasma/serum concentrations of SPMs can be meaningfully increased. Such a threshold may relate to the amount of EPA and DHA required to fuel SPM biosynthesis.

8. Summary and discussion

Although inflammation has a physiological role, unrestrained inflammation can be detrimental, causing tissue damage and leading to disease. Therefore, under normal circumstances inflammation is self-limiting with induction of active resolution processes. Central to these is the generation of SPMs from the bioactive n-3 PUFAs EPA and DHA (SPMs are also produced from docosapentaenoic acid). These SPMs include Rvs, protectins and maresins whose activities have been well described in numerous cell culture and laboratory animal models. The findings of these experiments suggest that inflammation can become pathological in the absence of endogenous synthesis of SPMs, that exogenous SPMs could be used therapeutically in conditions of adverse inflammation, and that the effects of SPMs may explain many of the observed effects of n-3 PUFAs. Hence there has been much interest in describing the presence of SPMs in blood, other fluids and tissues in human disease and in investigating whether providing EPA and DHA can increase SPM concentrations in humans. A number of studies report SPM concentrations in various pools in humans (Tables 1, 2 and 3). However, this is analytically challenging because of the low concentrations frequently seen. Hence many SPMs are reported as not detected in many studies (they may often be present but at concentrations below to level of detection) and there are wide variations within and between studies in the concentrations of SPMs reported that are not fully explained. These could relate to sample storage, sample processing and analytical differences. Nevertheless a number of SPMs have been reported in human plasma or serum in infants, children, healthy adults and individuals with various diseases (Table 1), as well as in human sputum, saliva, tears, breast milk, urine, synovial fluid and cerebrospinal fluid (Table 2) and in human adipose tissue, skeletal muscle, hippocampus, skin, placenta, lymphoid tissues and atherosclerotic plaques (Table 3). There may be differences in concentrations of some SPMs between males and females [94] and with

age [101] but these are not yet well explored. There are certainly differences in SPM concentrations between health and disease, as would be expected. Observations that SPM concentrations are lower in people with certain diseases than in healthy controls (or are lower in more severe compared to less severe disease) are consistent with the idea that many diseases, particularly those with a strong inflammatory component, are associated with a loss of, or at least a diminution in, pro-resolution factors including SPMs. Such a situation could be amenable to treatment with the n-3 PUFAs EPA and DHA, with the hydroxy-EPA and -DHA precursors to SPMs, or with SPMs themselves. However, there are also studies reporting higher concentrations of SPMs in some diseases than in healthy controls. These findings perhaps reflect an exaggerated inflammatory response which has induced resolution pathways as part of the normal attempt to restore homeostasis, but which fails. Such a failure could be because of an imbalance between the strong ongoing inflammation and an insufficiently strong resolution response. Alternatively, this situation may reflect a loss of responsiveness to pro-resolution signals either because of reduced receptor expression or failure of post-receptor signalling pathways. It is clear that more needs to be understood about the relationship of SPMs to human disease in order to fully evaluate appropriate targets and therapeutic options. It may be that in some situations therapeutic use of SPM precursors or of SPMs themselves may not be appropriate.

There has been significant interest in the ability of exogenous EPA and DHA to promote increased concentrations of SPMs. A number of studies report that plasma or serum concentrations of some SPMs are higher after a period of increased intake of EPA and DHA (Table 4). In adults, increases in concentrations of 18R-RvE1, 18R-RvE2, 18R-RvE3, RvD1 and RvD2 have been reported several times, but effects on AT-RvD1, other D-series Rvs, PD1 and MaR1 are less consistently seen (Table 4). There may be a threshold intake of n-3 PUFAs that is required before plasma/serum concentrations of SPMs can be meaningfully increased. Such a threshold may relate to the amount of EPA and DHA required to drive SPM biosynthesis. “Free” EPA and DHA are required as substrates for endogenous SPM biosynthesis. Most EPA and DHA in the bloodstream, in cell membranes and inside cells is esterified into phospholipids, triacylglycerols, cholesteryl esters and other complex lipids, although some non-esterified (“free”) EPA and DHA is found in the bloodstream. EPA and DHA are released from complex lipids, such as phospholipids, by lipase enzymes, such as phospholipases. These enzymes typically have a fairly broad specificity, for example for PUFAs at the *sn*-2 position of phospholipids. Hence, esterified EPA and DHA will comprise only part of the available substrate for lipase enzymes. Therefore, when the EPA and DHA

content of complex lipids, such as membrane phospholipids, is low, liberation of EPA and DHA is likely to be modest and other fatty acids like arachidonic acid, which is present in much higher amounts, are likely to be liberated preferentially. As EPA and DHA contents increase as a result of increasing oral intake, the likelihood for their liberation by lipases increases. Once PUFAs are liberated from their parent complex lipids they will compete for other enzymes like COXs and LOXs. Use of EPA and DHA in the COX pathways is enhanced in the presence of aspirin. Nevertheless, it is the availability of a range of competing substrate PUFAs for the enzymes involved in the pathways of SPM biosynthesis that likely establishes the threshold that is suggested by Figure 8B. At low intakes of EPA and DHA, the relative availability of these fatty acids in both free and esterified pools may be too low to promote significant endogenous SPM biosynthesis. Perhaps it is only when intakes are sufficient to create larger free and esterified pools of EPA and DHA that the pathway of SPM biosynthesis can be significantly enhanced. Even then, the increase in apparent SPM production (as reflected in the concentrations reported in the studies described in Table 4) is highly mediator-specific, with large increases reported for some, modest or no increases for some, and even decreases for some (Table 4). The widely reported increase in the concentrations of the immediate hydroxy fatty acid precursors to SPMs and the linear relationships of these concentrations to increased intake of EPA and DHA shown in Figures 6 and 7, support EPA and DHA provision as a strategy to promote endogenous synthesis of SPMs. However, the relationship of specific intakes of EPA and DHA to enhancement in the production of specific SPMs is not clear and needs a more thorough investigation. This is important because of the potential for EPA and DHA to be used more effectively in prevention and treatment of inflammatory conditions. If generation of SPMs represents an important mechanism of action of EPA and DHA, then more needs to be known about the most effective strategies by which EPA and DHA can increase SPM concentrations.

Conflicts of interest

PCC has received research funding from BASF AS and acts as a consultant to BASF AS, Smartfish, DSM, Cargill and Fresenius-Kabi.

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Figure captions

Figure 1. Schematic representation of self-limiting and chronic inflammation. Modified from Prostaglandins Leukotrienes and Essential Fatty Acids, Vol 131, J.K. Innes and P.C. Calder, Omega-6 fatty acids and inflammation, pp 41-48, Copyright 2018, with permission from Elsevier [7]. This version of the figure appeared in [12].

Figure 2. The role of n-3 polyunsaturated fatty acid (PUFA) derived specialised pro-resolving lipid mediators (SPMs) in resolving acute and chronic inflammation.

Figure 3. Outline of the pathway of synthesis of specialised pro-resolving mediators from eicosapentaenoic acid (EPA). COX, cyclooxygenase; CytP450, cytochrome P450; LOX, lipoxygenase; Rv, resolvin.

Figure 4. Outline of the pathway of synthesis of specialised pro-resolving mediators from docosahexaenoic acid (DHA). AT, aspirin-triggered; COX, cyclooxygenase; CytP450, cytochrome P450; LOX, lipoxygenase; MaR, maresin; P, protectin; Rv, resolvin.

Figure 5. Average concentrations of 11 specialised pro-resolving mediators in plasma or serum of healthy adult humans reported from different studies. All analyses used LC-MS/MS and reported concentration as weight/ml. Data are from [36-38,40,44,45,52,55,58 (plasma data only),59,60,66,70,72,73].

Figure 6. The relationship between increased EPA intake, the incorporation of EPA into different blood pools in healthy humans and the increase in plasma 18-hydroxy-EPA. Healthy human participants consumed fish oil providing different amounts eicosapentaenoic acid (EPA) and docosahexaenoic acid per week for one yr. EPA content of plasma phosphatidylcholine (closed circles), plasma non-esterified fatty acids (closed squares), mononuclear cells (open circles) and platelets (open squares) was determined by gas chromatography and is expressed as mean group difference in EPA as % of total fatty acids

from the placebo group at 12 months. These data are from [93]. Plasma 18-hydroxy-EPA concentrations (closed triangles) were determined by liquid chromatography-mass spectrometry and are expressed as mean group difference in nmol/l from the placebo group at 12 months. These data are from [104].

Figure 7. The relationship between increased DHA intake, the incorporation of DHA into different blood pools in healthy humans and the increase in plasma 17-hydroxy-DHA. Healthy human participants consumed fish oil providing different amounts eicosapentaenoic acid and docosahexaenoic acid (DHA) per week for one yr. DHA content of plasma phosphatidylcholine (closed circles), plasma non-esterified fatty acids (closed squares), mononuclear cells (open circles) and platelets (open squares) was determined by gas chromatography and is expressed as mean group difference in DHA as % of total fatty acids from the placebo group at 12 months. These data are from [103]. Plasma 17-hydroxy-DHA concentrations (closed triangles) were determined by liquid chromatography-mass spectrometry and are expressed as mean group difference in nmol/l from the placebo group at 12 months. These data are from [104].

Figure 8. The effect of oral n-3 PUFAs on plasma concentrations of specialised pro-resolving mediators in adult humans. A) Calculated average % change in plasma concentration of 5 specialised pro-resolving mediators in plasma of adult humans after consuming oral n-3 PUFAs reported from different studies. Data are combined from [35,37,38,41,63] (RvE1), [37,38,41,46,63] (RvE2 and RvE3), [37,38,41,42,43,63] (RvD1) and [37,38,41,42,43] (RvD2). Data are calculated as % difference between group average after and before n-3 PUFAs or, where before n-3 PUFA data were not available [46], between after n-3 PUFAs and values in the control group. B) Change in plasma concentrations of RvE1 (closed circles), RvE2 (open circles) and RvD1 (grey squares) according to increased daily intake of EPA (for RvE1 and RvE2) or DHA (for RvD1). Data are from [35,38,41,63] (RvE1), [38,41,46,63] (RvE2) and [38,41,42,43,63] (RvD1). All studies used LC-MS/MS for analysis. Data are calculated as % difference between group average after and before n-3 PUFAs or, where before n-3 PUFA data were not available [46], between after n-3 PUFAs and values in the control group.