Short-term n-3 fatty acid supplementation but not aspirin increases plasma proresolving mediators of inflammation

Anne Barden,1,2,* Emilie Mas,1,* Kevin D. Croft,*, Michael Phillips,† and Trevor A. Mori*

School of Medicine and Pharmacology,† Royal Perth Hospital Unit, and Western Australian Institute for Medical Research,1 University of Western Australia, Perth, Australia

Abstract  Resolution of inflammation is an active process involving specialized proresolving mediators (SPM) formed from the n-3 fatty acids. This study examined the effect of n-3 fatty acid supplementation and aspirin on plasma SPMs in healthy humans. Healthy volunteers (n = 21) were supplemented with n-3 fatty acids (2.4g/day) for 7 days with random assignment to take aspirin (300 mg/day) or placebo from day 5 to day 7. Blood was collected at baseline (day 0), day 5, and day 7. Plasma 18R/S-HEPE, E-series resolvins, 17R/S-HDHA, D-series resolvins, 14R/S-HDHA, and MaR-1 were measured by LC/MS/MS. At baseline concentrations of E- and D-series resolvins and the upstream precursors 18R/S-HEPE, 17R/S-HDHA ranged from 0.1nM to 0.2nM. 14R/S-HDHA was 3-fold higher than the other SPMs at baseline but MaR-1 was below the limit of detection. Supplementation with n-3 fatty acids significantly increased RvE1, 18R/S-HEPE, 17R/S-HDHA, and 14R/S-HDHA but not other SPMs. The addition of aspirin after 5 days of n-3 fatty acids did not affect concentrations of any SPM. N-3 fatty acid supplementation for 5 days results in concentrations of SPMs that are biologically active in healthy humans. Aspirin administered after n-3 fatty acids did not offer any additional benefit in elevating the levels of SPMs.—Barden, A., E. Mas, K. D. Croft, M. Phillips, T. A. Mori. Short-term n-3 fatty acid supplementation but not aspirin increases plasma proresolving mediators of inflammation. J. Lipid Res. 2014. 55: 2401–2407.

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There is increasing interest in the role of the specialized proresolving mediators (SPMs) that actively stimulate resolution of inflammation (1). In particular, recent attention has focused on those SPM derived from the long-chain n-3 fatty acids EPA and DHA. The SPMs formed from EPA are known as E-series resolvins, while those formed from DHA include protectins, D-series resolvins, and maresins (1). Studies have shown that SPMs increase with time during the inflammatory process (1, 2), acting on a number of G-coupled protein receptors, including the leukotriene B4 (LTB4) receptor to affect resolution of inflammation.

Metabolism of EPA by acetylated cyclooxygenase (COX)-2 or cytochrome P450 monoxygenase results in the formation of 18-hydroxyeicosapentaenoic acid (18-HEPE) that can be converted by 5-LOX to 5-hydroperoxy-18-hydroxyeicosapentaenoic acid (5-Hp-18-HEPE). The latter can undergo epoxidation and enzyme hydrolysis to yield resolvin E1 (RvE1), or reduction to yield resolvin E2 (RvE2). A third E-series resolvin, resolvin E3 (RvE3), is generated from EPA by the 12/15 LOX pathway (3). Studies using chiral chromatography indicate that acetylation of COX-2 by aspirin generates both 18S-HEPE and 18R-HEPE and their respective downstream E-series resolvins 18S-RvE1 and 18R-RvE1 (4).

Metabolism of DHA by either acetylated COX-2 or 15-LOX gives rise to 17-hydroperoxydocosahexaenoic acid (17-HpDHA) that is converted to 17-hydroxydocosahexaenoic acid (17-HDHA). 15-LOX gives rise to mainly 17S-HpDHA whereas acetylated COX-2 yields 17R-HpDHA (5). The action of 5-LOX on 17-HDHA generates the D-series resolvins RvD1–RvD6 (1). In the absence of 5-LOX, protectin D1 (PD1) is formed from 17-HpDHA. In macrophages, 12/15-LOX can act on DHA to form 14-hydroperoxydocosahexaenoic acid (14-HpDHA) that can be further metabolized to 14-hydroxydocosahexaenoic acid (14-HDHA) and the maresins (6).

Most of the research on the effects of SPMs has been in animal models. The resolvins and protectins have been...
shown to reduce arthritis (7), colitis (8), airway inflammation (9), ocular inflammation (10), and postoperative pain (11). However, the SPMs are likely to be important in a number of human conditions associated with inflammation. In this respect, biosynthesis of protectin D1 by eosinophils is reduced in patients with severe asthma (12). MaR-1 has been identified in synovial fluid of patients with rheumatoid arthritis (13) and RvD1 and RvD2 have been identified in adipose tissue of healthy patients, whereas PD1 and 17-HDHA are reduced in subcutaneous adipose tissue of patients with peripheral artery disease (14).

In a study of the human serum metabolome, the reported concentrations of RvE1 and RvD1 were in the nanomolar range (15). In healthy humans supplemented with n-3 fatty acids, plasma RvD1, RvD2, and the upstream precursors of D-series resolvins (17-HDHA) and E-series resolvins (18-HEPE) were reported at concentrations previously shown to be biologically active (e.g., 0.1–0.5 nM) (16). Together, these studies show that resolvins and protectins are present in biological fluids, cells, and tissues and are modified in situations associated with inflammation. The degree to which circulating levels of these mediators of inflammation resolution can be altered by increasing intake of their precursor n-3 fatty acids, and how the addition of aspirin affects their concentration, has not been addressed in humans. We hypothesized that aspirin consumption would result in increased plasma concentrations of SPMs in volunteers consuming n-3 fatty acids. The aim was to conduct a placebo controlled study of the effect of aspirin on plasma SPMs in healthy humans supplemented with n-3 fatty acids. A secondary aim was to examine the effect of aspirin on the chiral distribution of the upstream precursors of the E-series and D-series resolvins, 18-HEPE and 17-HDHA, respectively.

METHODS AND STUDY DESIGN

Recruitment of volunteers

Healthy men and women aged 40–70 years were recruited by newspaper advertisement from the general population. Volunteers gave informed written consent to participate in the study that was approved by the Human Research Ethics Committee of the University of Western Australia. The trial was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN1261000740009). Suitability for the study was assessed by telephone screening and a follow-up appointment in our research unit where the volunteers completed questionnaires relating to their lifestyle, diet, medication, and medical history. Height, weight, and blood pressure were measured and a fasted blood sample was obtained for measurement of glucose, triglycerides, total- and HDL-cholesterol. Exclusion criteria included allergy to aspirin, eating >1 fish meal/week, consuming fish oil capsules, smoking, drinking > 30 g of alcohol/day, or the presence of a diagnosed chronic disease.

The volunteers entered a study of n-3 fatty acid supplementation for 7 days during which all volunteers were instructed to take 4 × 1g capsules daily of fish oil (Omega Daily, Blackmores, Warrnambool, Victoria, Australia) (35% EPA + 25% DHA), that were consumed throughout the day with meals. The capsules provided 1.4g EPA/day and 1g of DHA/day. After 5 days, volunteers were matched for age and gender and randomly allocated to take either enteric-coated aspirin (3 x 100 mg tablets/day, Bayer, Pymble, Australia) or matched placebo (Bayer) with breakfast, lunch, and dinner for 2 days, in addition to the n-3 fatty acid supplements (Fig. 1). The participants, nursing, and laboratory staff were blinded to the treatment.

Fasted blood samples were collected prior to commencing n-3 fatty acid supplements (Day 0), after 5 days of n-3 fatty acids (Day 5), and at Day 7 (after 2 days of aspirin or placebo) (Fig. 1). Blood collection at day 7 was ~12 h after the last dose of aspirin/placebo.

Compliance with the treatment regime was checked by capsule count and plasma fatty acids (n-3 fatty acids) and by tablet count (aspirin/placebo).

Blood collection

Blood for measurement of plasma SPM was collected on ice into EDTA, reduced glutathione, and butylated hydroxytoluene. Samples were centrifuged at 4°C and plasma was stored at −80°C until analysis.

Measurement of SPM in plasma

Standards. 18R/S-hydroxy-5Z, 8Z, 11Z, 14Z, 16E-eicosapentaenoic acid (18R/S-HEPE), 17S-hydroxy-4Z, 7Z, 10Z, 13Z, 15E, 19Z-docosahexaenoic acid (17S-HDHA), 17R-hydroxy-4Z, 7Z, 10Z, 13Z, 15E, 19Z-docosahexaenoic acid (17R-HDHA), 7S,8R,17S-trihydroxy-4Z, 9E,13Z,15Z,19Z-docosahexaenoic acid (RvD1) 7S,8R,17R-trihydroxy-4Z, 9E,11E,13Z,15Z,19Z-docosahexaenoic acid (17R-RvD1), 7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z-docosahexaenoic acid (RvD2), MaR-1 (7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-docosahexaenoic acid) and leukotriene B_4-d_4 (LTB_4-d_4) were purchased from Cayman Chemicals (Ann Arbor, MI). 10R,17S-dihydroxy-4Z,7Z,11E,13E, 15Z,19Z-docosahexaenoic acid (PD1) standard was kindly provided as a gift by Professor Charles N. Serhan (Harvard Medical School, Boston, MA). 5S,18R-dihydroxy-6E,8Z,11Z,14Z,16E-eicosapentaenoic acid (RvE2), (17,18S-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentaenoic acid (RvE3) and 17,18R-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentaenoic acid (18R-RvE3) standards were a gift from Professor Makoto Arita (Department of Health Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan). 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid (RvE1) and (±)-14-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid (14R/S-HDHA) were kindly provided by Cayman Chemicals.

The assay was performed as previously described (16). Briefly, plasma (1 ml) and internal standard LTB_4-d_4 (500 pg for reverse phase chromatography or 1ng for chiral analysis) were acidified with 2 ml of 100 mM sodium acetate pH 3, applied to solid phase extraction cartridges (Bond Elut C18 500 mg, Agilent Technologies, Santa Clara, CA). The mobile phases were (A) 5 mM ammonium acetate (pH = 8.9) and methanol (50/50 v/v) for analysis by LC/MS/MS. The SPMs were quantitated using a Thermo Scientific TSQ Quantum Ultra Triple Quadrupole LC/MS System (ThermoFisher Scientific, Waltham, MA) equipped with an ESI source operated in negative ion mode. Instrument control and data acquisition were performed using the Xcalibur 2.0.7 software.

Separation of SPMs using reverse phase LC/MS/MS

LC was performed at ambient temperature on a Zorbax Eclipse XDB C18 column (2.1 × 100mm × 3.5um, Agilent Technologies, Santa Clara, CA). The mobile phases were (A) 5 mM...
Chiral analysis of R- and S- isomers of 18-HEPE and 17-HDHA

Chiral analysis was used to differentiate the R- and S- isomers of the upstream precursors of the E-series and D-series resolvins, 18-HEPE and 17-HDHA, respectively. 18R- and 18S-HEPE and 17R- and 17S-HDHA were separated by isocratic elution on a CHIRAL AD-RH column (2.1mm × 150 mm × 5 mm, Daicel Chemical Industries, Tokyo, Japan) using methanol/water (95/5; v/v) 0.01% acetic acid at a flow rate = 200 μL/min from 0 min to 6 min. SPMs in plasma were identified on the basis of having all of the following criteria: 1) retention time that matched the authentic standard SPMs; 2) multiple reaction monitoring (MRM) using two product ions identified from the standard SPM and optimized for collision energy; and 3) confirmation of retention time and MRM product ions in plasma with added standard SPMs (Table 1 and supplementary Figs. I–X). Mass spectra of the SPMs measured are given in supplementary Figs. XI–XV. The detection limit for 18-HEPE, 17-HDHA, and 14-HDHA was 10 pg/ml and the detection limit for other SPMs was 20 pg/ml.

TABLE 1.  Mass spectrum ions and collision energy use to identify SPM

| SPM          | Retention Time (min) | Parent Ion (m/z) | Product Ions (m/z) | Collision Energy (eV) for Product Ions |
|--------------|----------------------|------------------|--------------------|----------------------------------------|
|              |                      |                  | 1                  | 2                                      |
| 18R/S-HEPE   | 11.18                | 317              | 215                | 259                                    |
|              |                      |                  | 15                 | 14                                     |
| RvE1         | 1.38                 | 349              | 195                | 161                                    |
|              |                      |                  | 16                 | 17                                     |
| RvE2         | 5.07                 | 333              | 253                | 315                                    |
|              |                      |                  | 19                 | 15                                     |
| RvE3         | 5.72                 | 333              | 315                | 253                                    |
|              |                      |                  | 15                 | 19                                     |
| 18R-RvE3     | 7.08                 | 333              | 315                | 253                                    |
|              |                      |                  | 15                 | 19                                     |
| 17S-HDHA     | 15.51                | 343              | 201                | 245                                    |
|              |                      |                  | 14                 | 13                                     |
| RvD1         | 3.90                 | 375              | 215                | 233                                    |
|              |                      |                  | 18                 | 15                                     |
| 17R-RvD1     | 4.15                 | 375              | 215                | 233                                    |
|              |                      |                  | 18                 | 15                                     |
| RvD2         | 5.14                 | 375              | 175                | 259                                    |
|              |                      |                  | 25                 | 21                                     |
| PD1          | 8.24                 | 359              | 153                | 206                                    |
|              |                      |                  | 17                 | 18                                     |
| 14R/S-HDHA   | 16.21                | 343              | 161                | 233                                    |
|              |                      |                  | 13                 | 10                                     |
| MaR-1        | 8.29                 | 359.2            | 177                | 341                                    |
|              |                      |                  | 14                 | 15                                     |
| LTB4-d4 (IS) | 8.55                 | 339.2            | 197                | 321                                    |
|              |                      |                  | 19                 | 18                                     |
energy 14 and 13 eV, respectively. The LC/MS/MS chromatograms of 18R-HEPE, 18S-HEPE, 17R-HDHA, and 17S-HDHA are shown in supplementary Fig. XVI.

**Fatty acid analysis**

Plasma fatty acids were analyzed as previously described (17).

**Statistical analysis**

All data were checked for normality and where necessary transformed using a natural logarithm for parametric analysis. The effect of n-3 fatty acid supplementation on SPM concentrations was examined in the combined groups comparing day 5 to day 0 (Fig. 1) using linear mixed models with restricted maximum likelihood estimation. The additional effect of 2 days of treatment with aspirin or placebo on SPMs used a linear mixed model with restricted maximum likelihood estimation. This analysis compared the effect of aspirin with placebo on SPMs after 2 days (Day 7) and adjusted for between group differences in SPMs at randomization (Day 5).

**Statistical power**

Based on data from our previous study (16), we estimated that 10 subjects/group would give 80% power to detect a 50% difference in 18R/S-HEPE and 17R/S-HDHA at a significance level of $P=0.05$ using a linear mixed model.

**RESULTS**

Twenty-one men and women aged 53.7 ± 6.9 (mean ± SD) years with BMIs of 23.1 ± 2.5 kg/m² were recruited for the study. At baseline they had: cholesterol 5.1 ± 0.6 mmol/L, triglycerides 0.7 ± 0.2 mmol/L, HDL-cholesterol 1.7 ± 0.3 mmol/L and glucose 4.3 ± 0.6 mmol/L. The groups assigned to aspirin and placebo on day 5 were well matched for age and biochemistry. There were 4 males and 7 females in the aspirin group and 3 males and 7 females in the placebo group.

Compliance assessed by capsule count was 98% for n-3 fatty acid treatment and 99% for aspirin or placebo treatment. Plasma fatty acid analysis confirmed that after n-3 fatty acid supplementation EPA increased from 1.2 ± 0.1% to 4.0 ± 0.2% ($P=0.001$) and DHA increased from 2.4 ± 0.1% to 3.5 ± 0.2% ($P=0.001$).

**Effect of n-3 fatty acid supplementation on SPMs**

At baseline we detected measurable levels of all SPMs except PD1 and MaR-1 in plasma (Table 2). Because all participants took n-3 fatty acids, the effect of 5 days supplementation with n-3 fatty acids on SPMs was examined in the groups combined. After 5 days of n-3 fatty acid supplementation, there was a significant increase in the concentration of 18R/S-HEPE ($P<0.001$) (Table 2, Fig. 2), 17R/S-HDHA ($P=0.003$) (Table 2, Fig. 2), 14R/S-HDHA ($P=0.006$), and RvE1 ($P=0.003$) (Table 2). N-3 fatty acid supplementation for 5 days did not significantly alter the concentrations of other E-series resolvins, (RvE2, RvE3, and 18R-RvE3) or D-series resolvins (RvD1, 17R-RvD1, and RvD2) (Table 2).

**The effect of adding aspirin to n-3 fatty acid supplementation on SPMs**

The addition of aspirin for 2 days while taking n-3 fatty acid supplements did not significantly affect the concentrations of 18R/S-HEPE, 17R/S-HDHA, 14R/S-HDHA, or the E-series or D-series resolvins (Fig. 2, Table 2). Representative chromatograms at baseline (day 0), after n-3 fatty acid supplementation (day 5), and after aspirin (day 7) of 18R/S-HEPE, 17R/S-HDHA, and RvE1 are provided in supplementary Figs. XVII–XIX.

The effect of aspirin on the chiral distribution of the upstream precursors 18R-HEPE and 17-HDHA was examined after 2 days of aspirin (day 7) compared with day 5. The concentrations of the R- and S- isomers of 18R-HEPE and 17-HDHA were not significantly altered by aspirin (Table 3). However, the ratio of R- to S- isomers of 17-HDHA but not 18R-HEPE was significantly reduced by aspirin compared with placebo, $P=0.013$ (Fig. 3). Aspirin did not significantly alter the ratio of R- and S-isomers of RvE3 or RvD1 separated using reverse phase chromatography (data not shown).

**DISCUSSION**

Our study has shown for the first time that supplementing healthy men and women for 5 days with 2.4 g/day n-3 fatty acids significantly increased RvE1 and the upstream precursors of the E-series and D-series, 18R/S-HEPE and 17R/S-HDHA, respectively, as well as 14R/S-HDHA of the maresin pathway. Prior to n-3 fatty acid supplementation (day 0), the upstream precursors 18R/S-HEPE and 17R/S-HDHA, and RvE1, RvE2, RvE3, 18R-RvE3, RvD1, 17R-RvD1, and RvD2 were all quantified in human plasma with levels ranging between 0.1 nM and 0.2 nM. The concentration of 14R/S-HDHA was approximately 3-fold higher than that of 18R/S-HEPE or 17R/S-HDHA at day 0, and increased significantly with n-3 fatty acid supplementation. We were unable to detect MaR-1 in human plasma before or after n-3 fatty acid supplementation. We were also unable to detect PD1 in plasma, likely due the upstream precursor being utilized by 5-LOX to synthesize other D-series resolvins or because the plasma level of this SPM is lower than the detection limit of our instrument. Our study contrasts with that of Dawczynski et al. (18) who used a metabolomic approach to measure a large number of plasma eicosanoids by LC/ESI/MS/MS. The authors were unable to detect 17S-HDHA, RvE1, or RvD1 before or after 10 weeks of 3g/day of n-3 fatty acids provided by yogurt in hypertriglyceridemic patients (18). The different study findings may relate to the study population; our study recruited healthy volunteers. Additionally, the plasma n-3 fatty acid levels of the hypertriglyceridemic patients at baseline and postsupplementation were substantially lower than those of our healthy volunteers, thus potentially limiting substrate availability for SPM synthesis.

The addition of aspirin to the n-3 fatty acid supplement did not significantly alter the concentration of any SPM including the upstream precursors 18R-HEPE and 17-HDHA. These results agree with Oh et al. (19) who showed that total concentration of 18R-HEPE was not altered after a single dose of fish oil and aspirin.

Using a chiral column, we separated the individual isomers of 18R-HEPE and 17-HDHA. The concentrations of
the two isomers of 17-HDHA were similar after n-3 fatty acid supplementation and although total levels of 17-HDHA were not significantly altered by aspirin, the 17R-HDHA to 17S-HDHA ratio was reduced after aspirin. The concentration of the 18-HEPE isomers was similar after n-3 fatty acid supplementation. In contrast, in the study of Oh et al. (19), volunteers took aspirin (81 mg at 12 and 24 h) prior to a single dose of fish oil and collected blood after 3 h.

Another explanation for the divergent results could relate to the order in which n-3 fatty acids and aspirin were given. In our study, volunteers received n-3 fatty acids (2.4 g/day) for 5 days prior to randomization to aspirin (300 mg/day) or placebo for 2 days while continuing n-3 fatty acid supplementation. In contrast, in the study of Oh et al (19), volunteers took aspirin (81 mg at 12 and 24 h) prior to a single dose of fish oil and collected blood after 3 h. Another explanation for the divergent results could relate to compliance with aspirin intake in our study. However, tablet counts indicated 99% compliance for both aspirin and placebo tablets over the 2 days. In our study, the aspirin and placebo tablets were enteric-coated and 100 mg was taken with breakfast, lunch, and dinner (300 mg/day).

We chose this treatment regimen because aspirin administration throughout the day has been shown to be a more effective method of inhibiting COX-1 than a single dose of aspirin (20).

The fact that RvE1 and 17R/S-HDHA were significantly increased with n-3 fatty acids in our study is important because both of these SPMs have been shown to be biologically active. RvE1 has a broad range of beneficial effects that include inhibiting neuropathic and arthritic pain (7, 21), inhibiting renal fibrosis (22), protecting against the development of asthmatic airway inflammation (23), reducing reperfusion injury (24), and protecting against bone destruction in periodontitis (25). Stable analogs of RvE1 have been successful in trials for the treatment of dry eye in animal models (10) and a phase 2 clinical trial of synthetic RvE1 (RX-10045) has been completed in humans with allergic conjunctivitis. 17-HDHA has been shown to modulate macrophage function, alleviating experimental colitis (26), and has been implicated as important in antibody production, mediating B cell differentiation to antibody secreting cells (27).

The presence of RvE3 in human plasma may be an important finding given the fact that RvE3 resolvins, which

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**TABLE 2.** The effect of 5 days n-3 fatty acid supplements and the additional effect 2 days of aspirin on plasma SPM

| SPM (pg/ml) | n-3 Fatty Acids | P for Effect of 5 Days n-3 Fatty Acids (groups combined) | Randomization at Day 5 | n-3 Fatty Acids + Aspirin/Placebo Day 7 | P for Effect of 2 Days Aspirin

| Group | Day 0 | Day 5 | P | Placebo | Aspirin | P | Placebo | Aspirin | P |
|-------|-------|-------|---|--------|---------|---|--------|---------|---|
| 18R/S-HEPE | 86.3 (70.5,105.5) | 177.5 (135.7,232.1) | P < 0.001 | Placebo | 181.6 (149.4,229.7) | P = 0.31 |
| Group 2 | 89.9 (67.9,119.2) | 149.0 (107.7,206.2) | P = 0.61 | Placebo | 151.0 (119.1,191.1) |
| Group 1 | 37.8 (18.4,77.6) | 115.4 (57.3,232.1) | P = 0.003 | Placebo | 69.8 (39.8,122.4) | P = 0.35 |
| Group 2 | 49.9 (43.0,83.3) | 62.1 (32.4,119.1) | P = 0.91 | Placebo | 65.8 (28.4,154.5) |
| 18R-RvE3 | 67.9 (39.8,115.8) | 90.6 (61.7,133.0) | P = 0.29 | Placebo | 96.4 (64.0,145.3) | P = 0.56 |
| Group 2 | 100.3 (64.1,157.3) | 103.9 (58.5,184.6) | Placebo | 130.2 (64.5,263.2) |
| Group 1 | 25.9 (17.0,39.5) | 35.4 (21.2,59.3) | P = 0.23 | Placebo | 29.6 (19.3,45.4) | P = 0.73 |
| Group 2 | 36.0 (26.6,48.8) | 38.1 (22.0,55.8) | Placebo | 49.4 (26.4,92.3) |
| 17R/S-HDHA | 25.3 (14.3,45.9) | 34.9 (21.1,57.6) | P = 0.11 | Placebo | 24.2 (17.0,35.8) | P = 0.21 |
| Group 2 | 37.7 (26.7,53.1) | 45.1 (30.2,67.2) | Placebo | 45.1 (21.9,78.9) |
| Group 1 | 69.2 (49.5,96.7) | 118.9 (85.5,165.0) | P = 0.003 | Placebo | 102.6 (76.7,173.7) | P = 0.33 |
| Group 2 | 84.9 (46.8,157.4) | 90.7 (61.4,135.9) | Placebo | 89.6 (61.5,130.4) |
| RvE1 | 38.0 (28.2,51.2) | 44.2 (33.5,75.9) | P = 0.98 | Placebo | 36.5 (28.5,46.8) | P = 0.46 |
| Group 2 | 40.9 (29.5,56.8) | 37.5 (28.1,50.0) | Placebo | 42.9 (22.3,57.1) |
| 17R-RvD1 | 87.1 (72.9,104.3) | 71.7 (60.1,85.5) | P = 0.91 | Placebo | 80.2 (64.2,90.1) | P = 0.68 |
| Group 2 | 65.7 (55.8,77.5) | 77.9 (67.2,85.9) | Placebo | 70.9 (67.8,80.9) |
| RvD2 | 66.4 (57.2,83.7) | 70.2 (51.9,96.4) | P = 0.407 | Placebo | 68.8 (46.2,102.7) | P = 0.39 |
| Group 2 | 58.9 (46.9,74.1) | 76.7 (54.5,105.1) | Placebo | 59.7 (38.3,95.8) |
| 18R-SHDHA | 320.5 (168.7,616.5) | 708.4 (382.7,1311.6) | P = 0.006 | Placebo | 705.6 (358.1,1389.9) | P = 0.61 |
| Group 2 | 380.7 (186.0,779.7) | 398.6 (207.0,765.1) | Placebo | 335.9 (148.8,757.5) |

Values are mean and 95% confidence intervals. Groups 1 and 2 represent the placebo and aspirin groups, respectively, prior to randomization. Because all participants took n-3 fatty acids prior to randomization, mixed models examined the effect of n-3 fatty acids on SPMs (Groups 1 and 2 combined) at Day 5 adjusting for values at Day 0.

*aMixed models examined the additional effect of 2 days (Days 5 to Day 7) of aspirin compared with placebo on SPMs.
are synthesized by eosinophils via the 12/15 LOX pathway, have been shown to inhibit neutrophil infiltration in zymosan-induced peritonitis (3) and in vitro PMN chemotaxis (28). In a mouse model of endometriosis, EPA supple-
mentation reduced lesion formation and was associated with increased levels of RvE3 (29).

This study has shown that human plasma contains 14R/S-HDHA but not MaR-1, of the maresin family of
SPMs. Supplementation with n-3 fatty acids increased plasma 14R/S-HDHA by approximately 75%. Despite
the relatively high concentration of 14R/S-HDHA, we were unable to detect MaR-1 in human plasma after n-3 fatty
acid supplementation. The maresins are derived from macrophages during the resolution of inflammation (6).

Our study of n-3 fatty acids and aspirin was relatively short and was conducted in healthy volunteers who had no
underlying inflammatory conditions. Despite this, n-3 fatty acids resulted in significant increases in bioactive SPMs.
We did not observe additional synthesis of SPMs when

Aspirin was given in combination with n-3 fatty acids. Administration of n-3 fatty acids for 5 days prior to aspirin
may have stimulated SPMs maximally in these healthy subjects such that any effect of aspirin was minimal. It will be
important to study the effects and timing of n-3 fatty acids and aspirin on SPMs in patients with existing inflamma-
tory conditions. Taken together, our results suggest that n-3 fatty acid supplementation with EPA and DHA can ef-
ectively increase a number biologically active SPMs, which may in part explain the benefits of n-3 fatty acids in cardio-
vascular disease. The overall increase observed in the up-
stream precursors of the E- and D-series resolvins and
14R/S-HDHA with n-3 fatty acids supplementation could be a useful preventative strategy to limit the damage result-
ning from an impending inflammatory challenge.

Aspirin and matching placebo tablets were kindly donated by
Bayer, Pymble NSW, Australia. RvE2, RvE3, and 18RRvE3 standards
were a gift from Prof. Makoto Arita, University of Tokyo, Japan.

### TABLE 3. The effect of aspirin on isomers of 18-HEPE and 17-
HDHA measured using chiral chromatography during n-3 fatty acid
supplementation

| Isomer          | Aspirin (n = 11) | Placebo (n = 10) |
|-----------------|-----------------|-----------------|
| 18R-HEPE (pg/ml)| Day 5 n-3 FA    | 81.2 (56.3,106.1)| 82.4 (57.0,107.7) |
|                 | Day 7 n-3 FA ± Aspirin | 78.5 (58.2,98.7) | 84.1 (70.6,97.6) |
| 18S-HEPE (pg/ml)| Day 5 n-3 FA    | 90.4 (47.8,133.0)| 110.4 (62.1,158.7) |
|                 | Day 7 n-3 FA ± Aspirin | 84.3 (50.5,118.1) | 105.1 (67.4,142.8) |
| 17R-HDHA (pg/ml)| Day 5 n-3 FA    | 56.1 (36.3,75.9) | 72.2 (45.8,98.5) |
|                 | Day 7 n-3 FA ± Aspirin | 46.5 (29.9,62.9) | 61.8 (41.6,82.1) |
| 17S-HDHA (pg/ml)| Day 5 n-3 FA    | 54.3 (31.7,77.6) | 62.2 (41.5,82.9) |
|                 | Day 7 n-3 FA ± Aspirin | 54.2 (33.0,75.4) | 51.7 (35.0,68.4) |

Between group differences at Day 7 were compared using a mixed
model adjusting for levels at Day 5.
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