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The omega-3 fatty acids EPA and DHA decrease plasma F_2-isoprostanes: Results from two placebo-controlled interventions

EMILIE MAS[1], RICHARD J. WOODMAN[1], VALERIE BURKE[1], IAN B. PUDDEY[1], LAWRENCE J. BEILIN[1], THIERRY DURAND[2] & TREVOR A. MORI[1]

[1]*School of Medicine and Pharmacology, Royal Perth Hospital Unit, University of Western Australia, Perth, WA 6000, Australia, and* [2]*Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 CNRS/UM I/UM II, Faculty of Pharmacy, Montpellier, France*

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Abstract

Omega-3 (ω3) fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protect against cardiovascular disease. Despite these benefits, concern remains that ω3 fatty acids may increase lipid peroxidation. It has previously been shown that urinary F_2-isoprostanes (F_2-IsoPs) were reduced following ω3 fatty acid supplementation in humans. It is now determined whether EPA or DHA supplementation affects plasma F_2-IsoPs. In two 6-week placebo-controlled interventions, Study A: overweight, dyslipidaemic men; and Study B: treated-hypertensive Type 2 diabetic, patients were randomized to 4 g daily EPA, DHA. Post-intervention plasma F_2-IsoPs were significantly reduced by EPA (24% in Study A, 19% in Study B) and by DHA (14% in Study A, 23% in Study B) relative to the olive oil group. The fall in plasma F_2-IsoPs was not altered in analyses that corrected for changes in plasma arachidonic acid, which was reduced with EPA and DHA supplementation. Neither F_3- nor F_4-IsoPs were observed in plasma in both studies. These results show that in humans, EPA and DHA reduce *in vivo* oxidant stress as measured in human plasma and urine.

Keywords: *Isoprostanes, fish oil, inflammation*

Introduction

Oxidative stress, which is characterized by excessive production of reactive oxygen species and reduction of antioxidant defense mechanisms, has been implicated in the pathogenesis of cardiovascular disease [1]. Quantification of oxidative damage products in biological systems is important to understand the role of free radicals in disease states. Lipids, which undergo peroxidation, are major targets of free radical attack. Some of the chemically and metabolically stable oxidation products are useful *in vivo* biomarkers of lipid peroxidation. These include the isoprostanes.

Morrow et al. [2] reported that F_2-isoprostanes (F_2-IsoPs), a complex group of prostaglandin $F_{2\alpha}$-like compounds, were produced *in vivo* by non-enzymatic free radical peroxidation of arachidonic acid (AA, 20:4 ω6). F_2-IsoPs are thought to be formed from esterified arachidonate present in phospholipids [3,4] and are released as free acids by phospholipases [5,6], circulate in plasma and excreted in urine. A number of studies have shown that quantification of F_2-IsoPs represents the most reliable marker of *in vivo* lipid peroxidation and oxidative stress [1,7].

In recent years, additional related compounds have been discovered such as the neuroprostanes (F_4-IsoPs) [8–11] and F_3-IsoPs [12–14] derived from docosahexaenoic acid (DHA, 22:6 ω3) and eicosapentaenoic acid (EPA, 20:5 ω3), respectively. F_3- and F_4-IsoPs have been reported in animal tissue following high-dose supplementation with ω3 fatty acids [15,16] and in human urine following treatment with lipopolysacharide (LPS) [14].

Correspondence: Dr Emilie Mas, School of Medicine and Pharmacology, University of Western Australia, Medical Research Foundation Building (M570) Level 4, Rear 50 Murray Street PERTH Western Australia 6000. Tel: 61 8 9224 0259. Fax: 61 8 9224 0246. Email: emilie.mas@uwa.edu.au

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There is now considerable evidence that a diet rich in ω3 fatty acids derived from fish and fish oil, specifically EPA and DHA, has a beneficial effect on lowering cardiovascular and all-cause mortality [17–23]. EPA and DHA are physiologically the most important members of the ω3 class.

Despite the benefits associated with increased ω3 fatty acid consumption, there remains a theoretical concern that these fatty acids may increase the unsaturation index, leading to increased lipid peroxidation. Our previous data do not support the literature, suggesting adverse effects of ω3 fatty acids on lipid peroxidation. We showed that fish meals containing ω3 fatty acids and purified EPA or DHA given to Type 2 diabetic patients [24,25] and purified EPA or DHA given to overweight, mildly hyperlipidaemic men [26] decreased urinary F_2-IsoPs. We observed also that cord plasma and urinary F_2-IsoPs were reduced in infants whose mothers received 4 g daily of fish oil capsules during pregnancy [27].

We now aimed to determine whether purified EPA or DHA supplementation affects lipid peroxidation measured as changes in plasma IsoPs (F_2-, F_3- or F_4-IsoPs) and if this is dependent on AA availability in two placebo-controlled interventions in (i) mildly hyperlipidaemic men and (ii) treated hypertensive Type 2 diabetic patients.

Materials and methods

Participants

Study A: Hyperlipidaemic men. Mildly hypercholesterolemic but otherwise healthy, non-smoking men aged 20–65 years were recruited from the general community by media advertising [26]. Entry criteria included: serum cholesterol > 6 mmol/L, triglycerides > 1.8 mmol/L or both; body mass index (BMI; in kg/m^2) between 25–30; and no recent (previous 3 months) symptomatic heart disease, diabetes or liver or renal disease (plasma creatinine > 130 mmol/L). None of the subjects was regularly taking non-steroidal anti-inflammatory, anti-hypertensive or lipid-lowering drugs or other drugs known to affect lipid metabolism. All of the men had a usual weekly consumption of not more than one fish meal and drank < 210 mL ethanol/week. Fifty-nine of the 136 subjects screened satisfied the entry criteria.

Study B: Treated-hypertensive, Type 2 diabetic men and post-menopausal women

Fifty-nine non-smoking, treated-hypertensive, Type 2 diabetic men and post-menopausal women, aged 40–75 years, were recruited from the general community by media advertising [25]. All subjects were on anti-hypertensive therapy for a minimum of 3 months and showed previous evidence of diabetes (fasting glucose > 7.8 mmol/l or a 2 h post-prandial glucose > 11.1 mmol/l). Subjects were included if they were taking oral hypoglycaemic agents but not insulin. All subjects had: HbA_{1c} < 9%, BMI ≤ 35 kg/m^2, clinic systolic blood pressure > 115 mmHg and < 180 mmHg and diastolic blood pressure < 110 mmHg (measured on two separate days using a Dinamap 1846 SX/P monitor), serum cholesterol ≤ 7.0 mmol/l and triglycerides ≤ 7.5 mmol/l. All ate not more than two fish meals per week, were not regular consumers of fish oil supplements and drank < 40 g/d ethanol. Subjects were excluded if they had a recent (within 3 months) or past history of symptomatic heart disease, myocardial infarction, angina pectoris or stroke, recent (3 months) major surgery, significant liver or renal disease (plasma creatinine > 130 μmol/l), symptomatic autonomic neuropathy, were smokers or ex-smokers within the past 2 years or used non-steroidal anti-inflammatory drugs regularly. Women were not excluded from the study if they were taking hormone replacement therapy. Subjects on lipid-lowering drugs, aspirin or antioxidant vitamins were included in the study, but were asked not to change doses.

Studies design and intervention

In each study, during a 3-week baseline period, subjects were stratified by gender (diabetes patients), age and BMI and randomly assigned to 4 g/d of EPA, DHA or olive oil placebo capsules for 6 weeks. Capsules containing purified preparations of EPA ethyl ester (~96%), DHA ethyl ester (~92%) or olive oil (~75% oleic acid ethyl ester) were provided by the Fish Oil Test Materials Program and the U.S. National Institutes of Health. The vitamin E content of the oils was 1.7 mg/g α-tocopherol and 0.9 mg/g γ-tocopherol in the EPA capsules, 1.6 mg/g α-tocopherol and 0.9 mg/g γ-tocopherol in the DHA capsules and 1.0 mg/g α-tocopherol and 0.9 mg/g γ-tocopherol in the olive oil capsules.

All participants were instructed to maintain their usual diets, alcohol intakes and physical activities and not to make any changes to their lifestyle throughout the intervention period. At an initial interview, subjects were given written and verbal instructions by a dietician on how to keep diet records, with food weighed or measured. The same dietician monitored the dietary intake of all the volunteers at 2-week intervals and ensured that usual eating habits were maintained. A 3-day diet record (2 weekdays and 1 weekend day) was completed by the volunteers at baseline and intervention.

Alcohol intake, physical activity and any medications taken were monitored every second week during the intervention by using 7-day retrospective diaries. Weight was measured every second week with an electronic scale.

The ethics committee of the Royal Perth Hospital approved the studies and all subjects gave written informed consent. All procedures were in accordance with institutional guidelines.

Blood sampling

Blood samples for fatty acid and F_2-IsoPs analyses were collected at baseline and week 6 of the intervention. Fatty acids were prepared from EDTA plasma and analysed by gas chromatography (GC) as previously described [25,26].

Blood for plasma F_2-IsoPs was collected into EDTA and reduced glutathione, centrifuged at 4°C and the plasma stored at −80°C after the addition of butylated hydroxytoluene (BHT, 200 mg/ml) to prevent *ex vivo* oxidation.

Analysis of isoprostanes

Chemicals, reagents and chromatography. 15-F_{2t}-IsoP (8-iso-$PGF_{2\alpha}$ or $IPF_{2\alpha}$-III), 8-F_{2t}-IsoP-d_4 ($IPF_{2\alpha}$-IV-d_4) and 15-F_{3t}-IsoP (8-isoPGF_3 or $IPF_{3\alpha}$-III were purchased from Cayman Chemicals (Ann Arbor, MI) and used without further purification. Pentafluorobenzylbromide (PFBBr) and N,N-diisopropylethylamine (DIPEA) were purchased from Sigma Chemicals (St Louis, MO). The silylating agent *N,O-bis*-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA-TMCS, 99:1) (BSTFA) was purchased from Pierce Chemicals (Rockford, IL). Certify II cartridges were from Varian (Lake Forrest, CA). All solvents were of HPLC grade. The 4(*RS*)-F_{4t}-NeuroP (F_4-IsoP) was synthesized in our laboratory as previously described [28].

Measurement of isoprostanes

F_2-IsoPs were measured by gas chromatography-mass spectrometry (GC-MS) using electron capture negative chemical ionization and a modification of our previously reported method [29]. Briefly, plasma F_2-IsoPs with 8-IsoP-d_4 (5 ng) as internal standard, after basic hydrolysis, were acidified, prior to solid phase extraction on pre-washed Certify II cartridges (Varian). After washing with methanol/water and hexane/ethyl acetate the F_2-IsoPs were eluted with ethyl acetate/methanol, dried and derivatized.

Measurement of F_2-IsoPs, 8-IsoP-d_4, F_3-IsoPs and F_4-IsoPs monitored ions at *m/z* 569, 573, 567 and 593, respectively.

Statistical analysis

Diet records were analysed by using DIET/1 (version 4; Xyris, Brisbane, Australia), which is based on the Australian Food Composition Database NUTTAB 1995A (32). Data were analysed by using SPSS (SPSS Inc, Chicago) with general linear models to assess the effects of EPA or DHA relative to the olive oil group. Significance levels were adjusted for multiple comparisons by using the K matrix method. Values are reported as means ± SEMs.

Results

The baseline characteristics of the two study populations are given in Tables I and II and show there were no significant differences between intervention groups in any of the variables shown.

Study A: Hyperlipidaemic men

The mean plasma F_2-IsoPs for the whole group at baseline was 2279 ± 98 pmol/L and there were no significant differences between the groups. Plasma F_2-IsoPs at baseline were 2277 ± 151 pmol/L in the olive oil group (control), 2201 ± 135 pmol/L in the EPA group and 2368 ± 229 pmol/L in the DHA group.

Plasma F_2-IsoPs were decreased 24% by EPA (496.5 ± 98 pmol/L, $p < 0.0001$) and 14% by DHA (332 ± 127 pmol/L, $p = 0.009$) relative to the olive oil group (Figure 1A). After adjustment for baseline value post-intervention plasma F_2-IsoPs were 2311 ± 83 pmol/L in the olive oil group (control), 1757 ± 85 pmol/L and 1976 ± 90 pmol/L in the EPA and DHA groups, respectively. There was a small

Table I. Clinical characteristics of the groups at baseline in mildly hyperlipidaemic men.

	Olive Oil (control) ($n=20$)	EPA ($n=19$)	DHA ($n=17$)
Age (years)	48.4 ± 2.0	48.9 ± 1.7	49.1 ± 2.2
BMI (kg/m^2)	28.4 ± 0.5	29.0 ± 0.7	28.9 ± 0.7
Cholesterol (mmol/L)	6.47 ± 0.21	6.20 ± 0.20	6.18 ± 0.18
Triglycerides (mmol/L)	2.04 ± 0.19	2.01 ± 0.19	2.25 ± 0.40
24 h Blood pressure (mmHg)			
Systolic	119.1 ± 2.3	120.6 ± 2.6	124.2 ± 3.1
Diastolic	71.4 ± 1.4	73.6 ± 1.8	75.2 ± 1.6

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; BMI, body mass index; HbA_{1c}, glycated haemoglobin. Mean ± SEM. Differences between the groups in baseline measures were assessed by analysis of variance (ANOVA).

Table II. Clinical characteristics of the groups at baseline in treated-hypertensive Type 2 diabetic men and women.

	Olive Oil (control) ($n=16$)	EPA ($n=17$)	DHA ($n=18$)
Age (years)	61.5±1.9	61.2±2.3	60.9±1.9
Gender (M/F)	12/4	14/3	13/5
BMI (kg/m^2)	29.9±1.0	27.9±0.8	30.6±0.7
Cholesterol (mmol/L)	4.6±0.2	4.5±0.2	4.5±0.2
Triglycerides (mmol/L)	1.7±0.2	1.3±0.2	1.6±0.1
24 h Blood pressure (mmHg)			
Systolic	137.8±3.7	136.8±4.3	138.6±4.3
Diastolic	73.4±1.7	74.8±2.2	71.5±2.1
HbA_{1c} (%)	7.1±0.1	7.1±0.2	7.5±0.2
Fasting Glucose (mmol/L)	7.9±0.4	7.5±0.4	8.2±0.2

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; BMI, body mass index; HbA_{1c}, glycated haemoglobin. Mean ± SEM. Differences between the groups in baseline measures were assessed by analysis of variance (ANOVA).

non-significant change in plasma F_2-IsoPs in the olive group (31.5±83 pmol/L).

After adjustment for baseline value and plasma AA concentration, post-intervention measures showed a significant decrease in the EPA group (552±176 pmol/L, $p=0.003$) and the DHA group (333±159 pmol/L, $p=0.041$) relative to the olive oil group (Figure 1B).

F_3-isoprostanes and F_4-neuroprostanes were not detected in the plasma of patients taking either EPA or DHA

Study B: Treated-hypertensive, Type 2 diabetic men and post-menopausal women

The mean plasma F_2-IsoPs at baseline in the whole group was 1722±66 pmol/L and there were no significant differences between the three groups. Plasma F_2-IsoPs at baseline were 1655±71 pmol/L in the olive oil group (control), 1669±112 pmol/L in the EPA group and 1833±142 pmol/L in the DHA group. After adjusting for baseline values, there was a small non-significant change in plasma F_2-IsoPs in the olive group (126±164 pmol/L). Post-intervention plasma F_2-IsoPs were decreased 19% by EPA (221±132 pmol/L, $p=0.039$) and 23% by DHA (423±117 pmol/L, $p=0.011$) relative to the olive oil group (Figure 2A). Post-intervention plasma F_2-IsoPs were 1800±115 pmol/L in the olive oil group (control), 1463±111 pmol/L and 1378±109 pmol/L in the EPA and DHA groups, respectively, adjusting for baseline value.

After adjustment for baseline value and plasma AA concentration, there were non-significant decreases in the EPA group (237±197 pmol/L, $p=0.237$) and the DHA group (322±197 pmol/L, $p=0.109$) (Figure 2B).

F_3-IsoPs and F_4-IsoPs were not detected in the plasma of patients taking either EPA or DHA.

Discussion

We analysed plasma samples from two previously published placebo-controlled interventions of parallel design, in treated-hypertensive, Type 2 diabetic patients and in overweight, dyslipidaemic men, randomized to 4 g daily of purified EPA, DHA or olive oil for 6 weeks. Plasma F_2-IsoPs were reduced by 19% and 24% by EPA ($p < 0.05$) and 23% and 14% by DHA ($p < 0.05$) relative to the control group (olive oil) in treated-hypertensive, Type 2 diabetic patients and hyperlipidaemic men, respectively. In models that additionally adjusted for plasma AA concentration, plasma F_2-IsoPs remained significantly

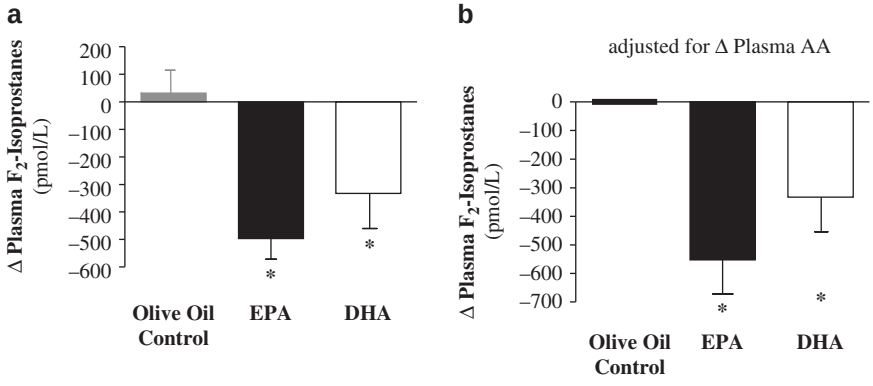

Figure 1. Change in plasma F_2-isoprostanes in overweight hyperlipidaemic men.

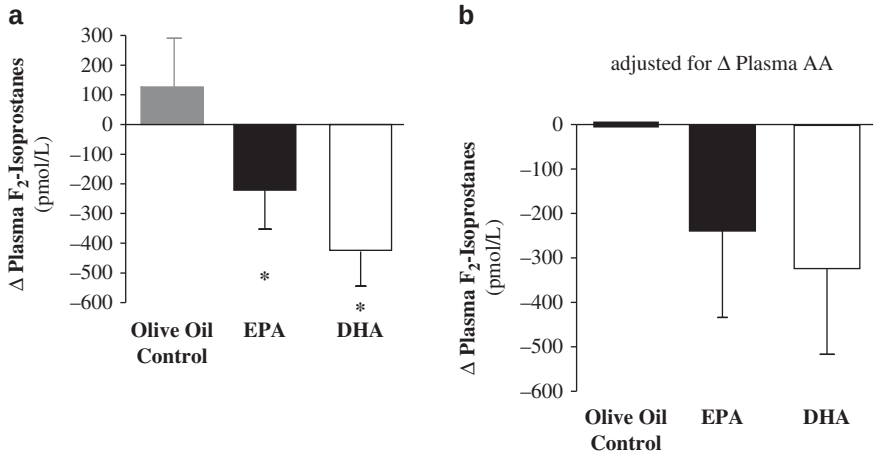

Figure 2. Change in plasma F_2-isoprostanes in hypertensive Type 2 diabetic patients.

decreased in hyperlipidaemic men supplemented with EPA or DHA relative to the control group. In treated-hypertensive, Type 2 diabetic patients the trend in decreased F_2-IsoP remained the same but did not reach significance. This is due likely to a greater variability in plasma levels of F_2-IsoPs in Type 2 diabetic patients and/or inadequate statistical power. We did not observe in either study the presence of F_3- or F_4-IsoPs.

These data support our previous reports of a reduction in urinary F_2-IsoPs in the same study populations. In overweight, mildly hyperlipidaemic men, supplementation decreased urinary F_2-IsoPs levels by 27% following EPA (1.24 nmol/24-h, $p < 0.0001$) and 26% following DHA (1.20 pmol/24-h, $p < 0.0001$), relative to an olive oil control group, after adjusting for baseline values [17,26]. In hypertensive Type 2 diabetic patients, we showed that urinary F_2-IsoPs were reduced 19% by EPA ($p=0.017$) and 20% by DHA ($p=0.014$), relative to an olive oil control group [25].

Our laboratory was the first to report that F_2-IsoPs are reduced after ω3 fatty acid intake. Our current data support previous reports in which we showed that fish meals providing ~3.6 g/day of ω3 fatty acids for 8 weeks to Type 2 diabetic patients, significantly ($p=0.013$) reduced urinary F_2-IsoPs by 20% [24]. This effect was independent of age, gender, body weight change and the increase in ω3 fatty acids or the fall in ω6 fatty acids in plasma, platelets and red blood cells. We have also shown cord plasma F_2-IsoPs were significantly lower ($p < 0.001$) in the offspring of women who had taken 4 g daily fish oil during pregnancy compared with those who took olive oil [27]. These differences were independent of red cell 20:4 ω6 levels. Urinary F_2-IsoPs corrected for creatinine excretion tended to be lower in infants whose mothers had taken fish oil ($p=0.06$) [27]. In each of our studies [24–27] the changes in F_2-IsoPs were unrelated to changes in EPA, DHA, AA, total ω3 or ω6 fatty acids.

Our findings are in accordance with other studies in which ω3 fatty acids have been supplemented. Quaggiotto et al. [30] showed that, compared to beef tallow, high doses of ω3 fatty acids during 6 weeks reduced plasma F_2-IsoPs after coronary occlusion in pigs ($n=10$). Similarly, Higdon et al. [31], in a blinded crossover trial in which post-menopausal women took 15 g high-oleate sunflower oil, high-linoleate safflower oil or fish oil rich in EPA and DHA for 5 weeks followed by a 7 weeks washout interval, demonstrated a fall in plasma F_2-IsoPs following ω3 fatty acids compared with diets enriched in oleate or linoleate. The differences, however, were eliminated when F_2-IsoPs were adjusted for plasma AA concentrations [31].

More recently, Nalsen et al. [32] showed in the KANWU study, a randomized multi-centre trial in healthy subjects supplemented with fish oil (3.6 g ω3 fatty acids/day containing 2.4 g EPA and DHA) or placebo capsules, ω3 fatty acids significantly reduced plasma F_2-IsoPs.

These above-mentioned studies showed that supplementation with ω3 fatty acids decreases F_2-IsoPs in both health and disease states. Furthermore, the lack of association with changes in fatty acids is noteworthy, in view of the fact that F_2-IsoPs are derived from free radical oxidation of AA, which is significantly reduced following ω3 fatty acids. Therefore, the changes in F_2-IsoPs most likely reflect a true reduction in oxidative stress, rather than resulting from a reduction in the supply of substrate.

Surprisingly, we did not observe F_3- or F_4-IsoPs in human plasma after supplementation with ω3 fatty acid. This contrasts with recently published data. Yin et al. [16] reported in a murine model of ovalbumin-induced lung inflammation that fish oil suppressed lung tissue F_2-IsoPs. Mice were fed with 4% (by weight) of olive oil (control group) or 2% or 4% of fish oil. Suppression of lung tissue F_2-IsoPs was dose-dependent. The authors also examined the effects of fish oil supplementation on EPA- and DHA-derived IsoPs in mouse lung tissue and observed a significant

increase of F_3-IsoPs and F_4-IsoPs (17-F_{4c}-neuroprostanes) in mouse lung tissue after 4% fish oil feeding. Gao et al. [15] have shown formation of F_3-IsoPs *in vitro* by oxidation of EPA and *in vivo* in tissue of rodent/mice fed EPA and administrated CCl_4. In heart tissue after 8 weeks of feeding by EPA they observed increased F_3-IsoPs and a decrease in F_2-IsoPs. They reported that levels of EPA in tissues from animals and humans are extremely low at baseline and F_3-IsoPs levels are below limits of detection (30 pg/g of tissue). Song et al. [14] observed an effect of fish oil on the formation of the F_3-IsoPs in urine of mice. In human urine they observed an acute inflammatory stimulus following LPS augments excretion of both urinary F_2-IsoPs and F_3-IsoPs.

It is difficult to explain why neither F_3- nor F_4-IsoPs were present in the plasma of the patients in our two interventions, in view of the above-mentioned studies. However, it is noteworthy that previous studies have observed F_3- and/or F_4-IsoPs in tissue and urine of animals and humans, respectively, but not in plasma. Moreover, these studies have employed an oxidative challenge to induce formation of the F_3-IsoPs and F_4-IsoPs. This is in contrast to our studies in which IsoPs were measured under basal conditions in individuals supplemented with ω3 fatty acids. Our finding is also likely related to the fact that there is a substantially higher content of cellular ω6 fatty acids, in particular AA, present compared with ω fatty acids.

Data in the literature regarding the potential oxidizability of EPA and DHA relative to AA is controversial and, in part, dependent on the experimental conditions employed. Recently, Richard et al. [33] reported evidence that a higher degree of unsaturation does not always equate with a greater susceptibility to oxidation. They suggested ω3 fatty acids might indirectly act as anti- rather than pro-oxidants in vascular endothelial cells, hence diminishing inflammation and, in turn, the risk of atherosclerosis and cardiovascular disease. Some studies indicate that the higher degree of unsaturation the greater the susceptibility to oxidation is not always true. Maziere et al. [34] compared the effects of ω6 and ω3 fatty acids incorporated into endothelial cells, with respect to cellular ability to oxidize LDL. They reported that ω3 fatty acids lowered TBARS production, superoxide anion secretion and LDL peroxidation as compared with ω6 fatty acids. Their interpretation was that ω3 fatty acids were less efficiently incorporated into cellular lipids, even though, after a similar percentage of polyunsaturated fatty acids (PUFA) incorporation, ω3 fatty acids still induced a less marked increase in LDL modification as compared with ω6 fatty acids. One explanation proposed by the authors is that the ω3 fatty acids, because of the position of their double bonds, are less susceptible to oxidative damage than the ω6 fatty acids. This hypothesis is further supported by a report from Visioli et al. [35], who demonstrated in an *in vitro* system the generation of oxidation products is not only related to the degree of unsaturation of fatty acids but also to the position of the double bonds. Yazu et al. [36], in aqueous micelles, reported a lower oxidizability of EPA than linoleate. In contrast, Davis et al. [37] suggested ω3 fatty acids are more oxidizable than ω6 lipids, and they can compete effectively for propagating peroxyls and alter the propagation/termination events, changing the consumption levels of ω3 fatty acids. Xu et al. [38] observed *in vitro* using a lipid bilayer model than the oxidation of fatty acids is dependent on the number of oxidizable bis-allylic $-CH_2-$ centres in the molecule.

The fall in F_2-IsoPs following ω3 fatty acids likely relates to their ability to attenuate inflammatory markers and inflammation [39]. The ω3 fatty acids are structurally and functionally distinct from the ω6 fatty acids. Typically, human inflammatory cells contain high proportions of the ω6 PUFA, AA and low proportions of ω3 PUFA. AA is the precursor of highly-active mediators of inflammation (2-series prostaglandins and 4-series leukotrienes). Levels of these lipid-derived regulators can be modulated by fish oil supplementation which may have an impact on inflammation. Feeding fish oil results in partial replacement of AA in inflammatory cell membranes by EPA, and this change leads to decreased production of AA-derived. This response alone is a potentially beneficial anti-inflammatory effect of ω3 PUFA [40,41].

High levels of TNF-α (tumour necrosis factor-α), IL-6 and increased cell-associated IL-1 have been implicated in causing some of the pathologic responses in patients with chronic inflammation conditions [42]. Production of IL-1, TNF-α and IL-6 and IL-8 has been decreased by EPA and DHA in cultured cells and rodent macrophage [42–45]. Mori et al. [25] showed in treated-hypertensive, Type 2 diabetic patients that the decrease of F_2-IsoPs was associated with changes in TNF-α independently of age, gender, BMI and treatment group. Both EPA and DHA reduced TNF-α. These results are in accordance with others studies where fish oil decreased production of TNF-α or IL-1 or IL-6 by mononuclear cells [46–49].

Additional support for anti-inflammatory actions of ω3 fatty acids has been provided by the recent discovery of the resolvins and protectins [50]. E-series resolvins are formed from EPA by a series of reactions involving COX 2 (acting in the presence of aspirin) and 5-lipoxygenase. D-series resolvins are formed from DHA by similar reactions or by a pathway involving lipoxygenase enzymes [51–53]. The term resolvins, resolution phase interaction products, was introduced to signify that the new structures are endogenous, local-acting mediators possessing potent anti-inflammatory and immunoregulatory properties [50,54]. These mediators could explain many of the anti-inflammatory actions of ω3 fatty acids.

Our present data have shown that EPA and DHA reduced plasma F_2-IsoPs in dyslipidaemic men and Type 2 diabetic individuals. These results confirm our previous findings that urinary F_2-IsoPs were reduced in the same study populations, as well as after consumption of fish and fish oils in other study groups. Furthermore, reduced plasma and urinary F_2-IsoPs are independent of changes in plasma AA concentration. We did not observe F_3- or F_4-IsoPs in the plasma of our study participants. The data, therefore, suggest 3 fatty acids reduce oxidative stress, which is likely related, at least in part, to their anti-inflammatory actions and the expected reduction in leukocyte activity. These findings give further support for supplementation of the diet with 3 fatty acids for cardiovascular risk reduction.

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