Sex differences in the plasma accumulation of oxylipins in response to supplemental n-3 fatty acids

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Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; SPM, specialized pro-resolving mediators

Oxylipins are oxygenated derivatives of polyunsaturated fatty acids (PUFAs). They can be formed by cyclooxygenase, lipoxygenase or cytochrome P450 omega-hydroxylase or epoxygenase enzymes or by non-enzymatic autooxidation [1,2]. Oxylipins include hydroperoxy-, hydroxy-, dihydroxy-, trihydroxy-, oxo- and epoxy-fatty acids [1,2]. Probably the most well-known oxylipins are the eicosanoids (prostaglandins, thromboxanes, leukotrienes and lipoxins) formed from arachidonic acid (20:4n-6) [1,3]. Eicosanoids are produced from other 20-carbon PUFAs including eicosapentaenoic acid (EPA; 20:5n-3) [1,3] and dihomo-gamma-linolenic acid (20:3n-6) [1,4]. Using these same enzymes, docosanoids are produced from the 22-carbon PUFAs adrenic acid (22:4n-6) [1], docosapentaenoic acid (both 22:5n-6 and 22:5n-3) [1,5] and docosahexaenoic acid (DHA; 22:6n-3) [1,6-8]. The DHA-derived oxylipins include protectins, maresins and D-series resolvins and their precursors [1]. Eighteen-carbon PUFAs can also give rise to oxylipins; these include hydroxy-, dihydroxy-, trihydroxy- and epoxy-derivatives of linoleic acid (18:2n-6) [1,9], gamma-linolenic acid (18:3n-3) [1], and alpha-linolenic acid (ALA; 18:3n-3) [1]. Other PUFAs such as stearidonic acid (18:4n-3) and the n-3 eicosatetraenoic acid (20:4n-3) are also substrates for oxylipin synthesis. Oxylipins are typically generated from PUFAs cleaved from cell membrane phospholipids, although phospholipid-bound oxylipins are also seen. The free oxylipins are often unstable with short half-lives. They can have significant biological activity acting via both cell membrane G protein coupled receptors and intracellular receptors [3] to affect many cell and tissue functions including those related to inflammation, immune responses, platelet reactivity and smooth muscle contraction [1,3]. Thus, the actions of oxylipins may explain some of effects of their parent PUFAs on physiological responses and health outcomes. The E-series resolvins produced from EPA and the D-series reolvins, protectins and maresins produced from DHA are well described to resolve inflammation [6-8] and have been collectively termed specialized pro-resolving mediators (SPMs). Oxylipins are produced in increased amounts when cells or tissues are stimulated, and many oxylipins can be measured circulating in the human bloodstream [10,11]. Oxylipins formed from linoleic acid are present at the highest concentrations in human blood plasma and serum [10,11]. Since oxylipins are produced from PUFA substrates, it is likely that the generation of oxylipins is, at least partly, related to the amount of substrate PUFA available. Accordingly, production of prostaglandin E_2 by both rat and human inflammatory cells has been linearly related to cell membrane arachidonic acid content [12,13]. Hence, increasing or decreasing cell membrane PUFA content could be a strategy to control the production of desirable or undesirable oxylipins. In this context it has been demonstrated that increasing the intake of the n-3 PUFAs EPA and

DHA results in increased blood plasma concentrations of many EPA- and DHA-derived oxylipins (reviewed in [14]). Ostermann et al. reported clear linear dose response relationships between supplemental EPA and DHA intake and plasma concentrations of numerous oxylipins derived from those two fatty acids [15]. Several factors including sex [16] and age [17,18] are reported to influence plasma and cell levels of PUFAs. Thus, these same factors could influence oxylipin production and concentrations. Relatively little is known about this.

In the current issue of *Journal of Nutrition*, Gabbs et al. report the effect of increased intake of ALA or DHA on the time course of changes in plasma oxylipin concentrations in humans and whether this differs between males and females [19]. They gave healthy males or females aged 19 to 34 years supplements providing ~4 g/day of either ALA or DHA for 28 days in a cross-over study design with a 6-week wash-out between phases; the DHA group also consumed 0.88 g/day EPA from the DHA supplement. Oxylipin concentrations were reported at days 0, 1, 3, 7, 14 and 28 of each period. Concentrations of 16 out of 62 oxylipins measured, including 5 out of 12 DHA-derived oxylipins, were higher in females than males, although plasma PUFAs were not different between sexes. ALA supplementation more than doubled plasma ALA but did not affect ALA-derived oxylipins concentrations. DHA supplementation more than doubled plasma DHA and increased plasma concentrations of several DHA-derived oxylipins including 8-, 11-, 14- and 20-hydroxy-DHA, 19,20-dihydroxy-docosapentaenoic acid (n-3) and 19,20-epoxy-docosapentaenoic acid (n-3). Increases in some of these oxylipins were observed at day 1 and 11 out of 12 DHA-derived oxylipins reached their plateau concentrations by day 7. In this study, plasma EPA more than doubled with DHA supplementation and several EPA-derived oxylipins (including 5-, 12-, 15- and 18-hydroxy-EPA) were increased with a similar time course to that seen with the DHA-derived oxylipins. This may be because the DHA supplement also contained some EPA. In addition, DHA supplementation has often been demonstrated to result in EPA enrichment in blood lipids and blood cells. This has usually been considered to result from the so-called “retro-conversion” of DHA to EPA, although a recent study using stable isotope tracing of the fate of DHA indicates that such retro-conversion is minimal in humans [20]. Those authors suggest that EPA enrichment with DHA supplementation is due to decreased metabolism of EPA. Several plasma oxylipins reached their plateau concentration earlier in females than males. Unexpectedly perhaps, DHA supplementation also resulted in higher concentrations of 4 arachidonic acid-derived oxylipins as well as one oxylipin derived from each of linoleic acid and dihomo-gamma-linolenic acid than seen with ALA. Nevertheless, DHA did not alter plasma concentrations of linoleic or arachidonic acids, although it did lower the concentration of

dihomo-gamma-linolenic acid. These findings suggest that substrate concentration may not be the only determinant of plasma oxylipin concentrations, although it is important to keep in mind that the oxylipins in plasma have been produced by cells (e.g. endothelial cells, leukocytes, platelets) and this study does not report on cell PUFA content. Nevertheless, it would be unexpected for DHA to increase cell linoleic and arachidonic acid contents. Thus, the explanation for the higher concentrations of a small number of n-6 PUFA-derived oxylipins after DHA compared to after ALA is not clear, although these effects may indicate an effect of DHA on metabolism of n-6 PUFAs.

The main findings of this study that several n-3 PUFA-derived oxylipins increase in plasma over the course of a few days of supplementation and that the rise is faster in females are of interest. The similar time course of appearance of EPA and DHA and their respective oxylipins in plasma establishes a clear substrate-product relationship as suggested by the dose-response data published by other others previously [15]. However, the faster accumulation of oxylipins in plasma in females than males without an evident difference in accumulation of precursor PUFAs between the sexes suggests that the substrate-product relationship has some subtleties. Variation in PUFA handling and metabolism between males and females and the roles of sex-differences in diet, body size, body composition, physical activity and (sex) hormone status in determining such variation need exploration. This is important because many of the oxylipins reported by Gabbs et al. [19] have biological activity and several are precursors to highly active SPMs. Therefore, understanding how it is that females respond more quickly to the n-3 PUFA-driven changes in plasma oxylipins may help refine the therapeutic use of n-3 PUFAs.

Strengths of the study of Gabbs et al. [19] are its cross-over design and the use of multiple sampling times. In addition, compliance to supplement intake is reported to be high (93%). One weakness of the study of Gabbs et al. [19] is that the sample size was small, with 6 males and 6 females being studied. These participants were young adults with normal blood triglyceride concentrations and a normal body mass index. Responses to PUFAs and oxylipin production may differ with age and with body fatness and therefore the effects observed in this study cannot be extrapolated to other sub-groups of the population which might be targets for the effects of n-3 PUFAs such as older adults or people with obesity. Plasma linoleic acid and arachidonic acid concentrations did not change with DHA supplementation which is unusual given the dose of n-3 PUFAs used; a decrease in both n-6 PUFAs might be expected at such a dose. Plasma triglyceride concentration was not affected in the DHA group; this is surprising as ~4 g/day DHA + 0.88 g/day EPA is a triglyceride lowering dose of these PUFAs. However,

plasma triglyceride concentration was only 1 mmol/L in these participants and this may be below the concentration at which n-3 PUFAs can have a triglyceride lowering effect.

In conclusion., Gabbs et al. [19] present novel and important data on the time course of plasma oxylipin concentrations in males and females in response to increased intake of ALA or DHA. ALA was largely without effect while DHA (+ EPA) increased EPA- and DHA-derived oxylipins over the course of several days with a faster change being evident in females. These findings contribute to our understanding of n-3 PUFA actions in humans and importantly they raise a number of questions for future interrogation of this dataset and for future research.

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PCC, as sole author, designed and wrote the manuscript and is responsible for the final content.

Conflicts of interest

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