

**Is increasing microbiota diversity a novel anti-inflammatory action of
marine n-3 fatty acids?**

Philip C. Calder^{1,2}

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

²National Institute of Health Research Southampton Biomedical Research Centre,
University Hospital Southampton National Health Service Foundation Trust and University
of Southampton, Southampton, United Kingdom

Address for correspondence: Dr P.C. Calder, Human Development and Health, Faculty of
Medicine, University of Southampton, IDS Building, Mailpoint 887 Southampton General
Hospital, Tremona Road, Southampton SO16 6YD, United Kingdom

Tel: + 44 2381205250

Email: pcc@soton.ac.uk

Running title: Marine n-3 fatty acids and microbiota diversity

Funding: The author has received no funding related to this manuscript.

Keywords: Omega-3; Eicosapentaenoic acid; Prostaglandin E₂; Colon; Inflammation

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PG, prostaglandin.

In the current issue of Journal of Nutrition, Djuric *et al.* describe a relationship between increased diversity of the gut microbiota and the anti-inflammatory effect of supplemental n-3 fatty acids, assessed as colonic prostaglandin (PG) E₂ concentrations [1]. The findings, which must be regarded as preliminary and requiring confirmation, suggest a new mechanism by which the marine n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) dampen intestinal inflammation. Importantly, rather than sampling fecal microbiota, Djuric *et al.* [1] sample the microbiome of colonic mucosal biopsies and of stool brushings from an adjacent site within the colon lumen some distance away from the anus. The bacteria present in the colonic mucosa and nearby stool are known to differ [2,3].

There are a number of well described, perhaps interacting, mechanisms by which EPA and DHA exert anti-inflammatory actions [4,5]. The oldest described mechanism is the partial replacement of arachidonic acid in the membranes of cells involved in inflammatory responses resulting in decreased availability of substrate for production of pro-inflammatory eicosanoids like PGE₂ and leukotriene B₄ (see [4,5]). The other main mechanism involves EPA and DHA interfering in the activation of the pro-inflammatory transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (aka NF-κB) resulting in decreased expression of genes encoding pro-inflammatory cytokines, adhesion molecules and enzymes (see [4,5]). This mechanism seems to result from several different actions of EPA and DHA including modulation of the formation of pro-inflammatory lipid rafts in response to inflammatory stimuli, activation of peroxisome proliferator activated receptor γ, and signalling through cell surface G-protein coupled receptor 120 (see [4,5]). The resulting reduction in inflammation is considered to be central to the role of marine n-3 fatty acids in preventing atherosclerosis [6] and in reducing pain and other symptoms in patients with rheumatoid arthritis [7,8]. There is inconsistent evidence of benefit of EPA and DHA in inflammatory bowel diseases [9] and in asthma and other allergic diseases [10]. More recently, the role of EPA and DHA as substrates for the generation of lipid mediators that actively resolve inflammation has been well described, mainly in pre-clinical studies in model systems [11], indicating that these fatty acids possess both anti-inflammatory and pro-resolving activities. Irrespective of the mechanism of action involved, the increased presence of EPA and DHA in the bloodstream and in the membranes of the cells involved is essential for the described effects to occur. Human studies have reported the linear dose-response relationship between increased intake of EPA and DHA, usually from supplements, and the increased appearance of these fatty acids in blood lipids [12-15] and in circulating

mononuclear cells [14,15] and neutrophils [16]. The enrichment of cell membranes with
60 marine n-3 fatty acids is accompanied by a decline in content of arachidonic acid and this
decline appears to be strongly inversely related to the intake of n-3 fatty acids [14,16,17].
These relationships are of biological significance. For example, Rees *et al.* [14] reported: “a
significant positive relation between PGE₂ production by lipopolysaccharide-stimulated
mononuclear cells and mononuclear cell phospholipid arachidonic acid content and a
65 significant negative relation between PGE₂ production by lipopolysaccharide-stimulated
mononuclear cells and mononuclear cell phospholipid EPA content”, “a significant positive
relation between PGE₂ production and the ratio of arachidonic acid to EPA in mononuclear
cell phospholipids” and “a significant negative relation between the change in PGE₂
production and the change in mononuclear cell phospholipid EPA content”. These relations
70 indicate a close link between the presence of both arachidonic acid and EPA in cell
membrane phospholipids and the ability of those cells to produce inflammatory mediators
and furthermore that one strategy to regulate inflammation is to modulate the amounts of
arachidonic acid and EPA in cell membrane phospholipids. Djuric *et al.* [1,18] set out to
decrease colonic mucosal PGE₂ production by 50%, since a reduction of this extent was
75 demonstrated to significantly affect colonic neoplasia in animal models (see [18]). Masoodi
et al. [19] reported 63% lower PGE₂ in uninflamed compared with inflamed colonic mucosa
from patients with Crohn’s Disease. Djuric *et al.* used serum fatty acids to report on
arachidonic acid and EPA levels combined with a personalized dosing approach with an
EPA-rich supplement [18]. The basis for this was both animal and human data relating the
80 ratio of EPA to arachidonic acid in serum to colonic PGE₂ concentration (see [18]). They
identified that a serum EPA to arachidonic acid ratio of about 1 was associated with a 50%
reduction in colonic PGE₂ concentration, and that achieving a serum ratio of 1 required an
individual dosing regimen of between 2.8 and 8.8 g EPA+DHA/day (mean 5.5 g/day) using a
supplement with an EPA to DHA ratio of about 3. These data may be compared with those of
85 Rees *et al.* [14] using 4.95 g EPA+DHA/day with an EPA to DHA ratio of 4.5 in the
supplement. After 12 wk, this resulted in a mean plasma phospholipid EPA to arachidonic
acid ratio of 0.78 in young men (mean age 24 y) and of 1.22 in older men (mean age 60 y)
and a mean reduction of lipopolysaccharide-induced PGE₂ production from mononuclear
cells of 47% for younger men and 50% for older men. Curiously, Djuric *et al.* have not
90 reported colonic mucosa fatty acids in detail in either of their publications [1,18]; it would be
very informative to know the relationships of the different fatty acids and fatty acid ratios
between serum and colonic mucosa. Others have reported higher arachidonic acid, lower

EPA and a higher ratio of arachidonic acid to EPA in inflamed compared with non-inflamed colonic mucosa from patients with Crohn's Disease [20].

95 In their earlier paper, Djuric *et al.* reported a mean 45% reduction in colonic mucosa PGE₂ concentration after 12 wk supplementation with the personalized dose of n-3 fatty acids [18]. In this new paper [1], they report data from the 47 healthy men and women who underwent a colonic mucosal biopsy and luminal stool brushing at study entry and exit, after 12 wk of personalized n-3 fatty acid supplementation. Subjects were aged 25 to 75 y and had
100 a body mass index of between 18 and 40 kg/m². The samples were collected 20 to 25 cm from the anal sphincter. Bacterial diversity was assessed using 16S rRNA sequencing. N-3 fatty acids had little effect on intestinal bacteria with no changes in relative abundance of major phyla or families or in diversity indices. This is generally consistent with the recent findings of Watson *et al.* [21] with marine n-3 fatty acids in healthy subjects: they reported
105 no significant changes in α or β diversity or phyla composition with n-3 fatty acid supplementation. However, Watson *et al.* [21] did find an increased abundance of several genera, including bifidobacteria, roseburia and lactobacilli with n-3 fatty acid intervention, although these changes did not correlate with erythrocyte n-3 fatty acid incorporation. Djuric *et al.* [1] found that n-3 fatty acids increased the dis-similarity index between the microbiome
110 in colonic mucosa and luminal brushings. A small group of subjects with high *Prevotella* abundance at study entry were resistant to the anti-inflammatory effects of n-3 fatty acids. In regression analyses, increases in bacterial diversity in luminal brushings, but not in colonic mucosa, were predictors of lower colonic PGE₂ concentrations. Changes in luminal brushing bacterial diversity contributed to 6 to 8% of the inter-individual variation in the change in
115 colonic PGE₂ concentration. The suggestion is that n-3 fatty acids increase diversity in stool microbiota and that this affects colonic mucosal inflammation, as evidenced by lower PGE₂ concentrations. An increased colonic mucosa ratio of EPA to arachidonic acid was also associated with decreased colonic mucosa PGE₂ concentration, as would be expected, but this did not influence the association of stool bacterial diversity with colonic PGE₂ concentrations
120 suggesting that the effect of the bacterial diversity is independent of the colonic mucosa fatty acid changes.

Where does this new research leave us? The personalization of n-3 fatty acid dosing against a physiological outcome is an intriguing, yet effective, approach. Marine n-3 fatty acids might affect diversity of stool microbiota to create a less inflammatory environment for
125 the colonic mucosa. Yet effects of n-3 fatty acids on the microbiota are small. Fat digestion and absorption are efficient, at least in most healthy human subjects, and it is unlikely that

much EPA and DHA pass through to the lower colon to act as a prebiotic or metabolic substrate. Perhaps a change in systemic inflammation resulting from increased presence of EPA and decreased presence of arachidonic acid is influencing the luminal microbiome. The study by Djuric *et al.* [1] is important because it highlights a possible new mechanism of action of marine n-3 fatty acids that could be important in promoting health and treating disease. However, the number of subjects investigated is fairly small, the biological effects are small despite the high intakes of EPA used, there is no control of the diet of the participants, and there is no control group with which to compare the temporal changes that are observed in the stool brushing microbiome.

References

1. Djuric Z, Bassis CM, Plegue MA, Sen A, Turgeon DK, Herman K, Young VB, Brenner DE, Ruffin MT. Increases in colonic bacterial diversity after omega-3 fatty acid supplementation predict decreased colonic prostaglandin E₂ concentrations in healthy volunteers. *J Nutr* 2019;in press.
2. Ringel Y, Maharshak N, Ringel-Kulka T, Wolber EA, Sartor RB, Carroll IM. High throughput sequencing reveals distinct microbial populations within the mucosal and luminal niches in healthy individuals. *Gut Microbes* 2015;6:173-181.
3. Yasuda K, Oh K, Ren B, Tickle TL, Franzosa EA, Wachtman LM, Miller AD, Westmoreland SV, Mansfield KG, Vallender EJ, et al. Biogeography of the intestinal mucosal and luminal microbiome in the Rhesus macaque. *Cell Host Microbe* 2015;17:385-391.
4. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta* 2015;1851:469-484.
5. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochem Soc Trans* 2017;45:1105-1115.
6. Calder PC, Yaqoob P. Omega-3 (n-3) fatty acids, cardiovascular disease and stability of atherosclerotic plaques. *Cell Mol Biol* 2010;56:28-37.
7. Abdulrazaq M, Innes JK, Calder PC. Effect of w-3 polyunsaturated fatty acids on arthritic pain: a systematic review. *Nutrition* 2017;39-40:57-66.
8. Senftleber NK, Nielsen SM, Andersen JR, Bliddal H, Tarp S, Lauritzen L, Furst DE, Suarez-Almazor ME, Lyddiatt A, Christensen R. Marine oil supplements for arthritis pain: A systematic review and meta-analysis of randomized trials. *Nutrients* 2017;6:42.

- 160 9. Calder PC. Fatty acids and immune function: relevance to inflammatory bowel diseases. *Int Rev Immunol* 2009;28:506-534.
10. Miles EA, Calder PC. Can early omega-3 fatty acid exposure reduce risk of childhood allergic disease? *Nutrients* 2017;9:784.
11. Serhan CN. Discovery of specialized pro-resolving mediators marks the dawn of
165 resolution physiology and pharmacology. *Mol Aspects Med* 2017;58:1-11.
12. von Schacky C, Fischer S, Weber PC. Long term effects of dietary marine ω -3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *J Clin Invest* 1985;76:1626-1631.
13. Katan MB, Deslypere JP, van Birgelen APJM, Penders M, Zegwaard M. Kinetics of the
170 incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes and adipose tissue: an 18 month controlled study. *J Lipid Res* 1997;38:2012-2022.
14. Rees D, Miles EA, Banerjee T, Wells SJ, Roynette CE, Wahle KWJW, Calder PC. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *Am J Clin Nutr* 2006;83:331-342.
- 175 15. Browning LM, Walker CG, Mander AP, West AL, Madden J, Gambell JM, Young S, Wang L, Jebb SA, Calder PC. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. *Am J Clin Nutr* 2012;96:748-758.
16. Healy DA, Wallace FA, Miles EA, Calder PC, Newsholme P. The effect of low to
180 moderate amounts of dietary fish oil on neutrophil lipid composition and function. *Lipids* 2000;35:763-768.
17. Walker CG, West AL, Browning LM, Madden J, Gambell JM, Jebb SA, Calder PC. The pattern of fatty acids displaced by EPA and DHA following 12 Months supplementation varies between blood cell and plasma fractions. *Nutrients* 2015;7: 6281-6293.
- 185 18. Djuric Z, Turgeon DK, Sen A, Ren J, Herman K, Ramaswamy D, Zhao L, Ruffin MT 4th, Normolle DP, Smith WL, Brenner DE. The anti-inflammatory effect of personalized omega-3 fatty acid dosing for reducing prostaglandin E2 in the colonic mucosa is attenuated in obesity. *Cancer Prev Res (Phila)* 2017;10:729-737.
19. Masoodi M, Pearl DS, Eiden M, Shute JK, Brown JF, Calder PC, Trebble TM. Altered
190 colonic mucosal polyunsaturated fatty acid (PUFA) derived lipid mediators in ulcerative colitis: new insight into relationship with disease activity and pathophysiology. *PLoSOne* 2013;8:e76532.

20. Pearl DS, Masoodi M, Eiden M, Brümmer J, Gullick D, McKeever TM, Whittaker MA, Nitch-Smith H, Brown JF, et al. Altered colonic mucosal availability of n-3 and n-6 polyunsaturated fatty acids in ulcerative colitis and the relationship to disease activity. *J Crohn's Colitis* 2014;8:70-79.
21. Watson H, Mitra S, Croden FC, Taylor M, Wood HM, Perry SL, Spencer JA, Quirke P, Toogood GJ, Lawton CL, et al. A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. *Gut* 2018;67:1974-1983.