Fishing for resolution

Philip C. Calder[1,2]

[1]School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, United Kingdom
[2]National Institute for Health Research Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton SO16 6YD, United Kingdom

Address for correspondence: 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; pcc@soton.ac.uk

Funding disclosure: The research described in this article received no funding.

Conflict of interest: PCC has received advising and speaking honoraria from Fresenius Kabi, B. Braun and Baxter Healthcare, produces of lipid emulsions for use in intravenous nutrition support.

In this issue of the *American Journal of Clinical Nutrition*, Noureddine et al. (1) report the concentrations of bioactive lipid mediators in the liver and spleen of mice receiving intravenous lipid emulsions based on soybean oil or fish oil for a period of 7 days. This work is supported by in vitro experiments where the concentrations of bioactive lipid mediators were measured in human $CD4^+$ T cells and $CD14^+$ monocyte-derived macrophages incubated with the lipid emulsions and stimulated with common agonists. The focus of the research is the so-called specialized pro-resolving mediators (SPMs) produced from the omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Lipid emulsions are used as part of intravenous nutrition support in order to provide calories, essential fatty acids, and fat-soluble vitamins (2,3). The traditionally used lipid emulsion is based on soybean oil and hence has a high content of the omega-6 PUFA linoleic acid. There has been a view that this emulsion contains an unbalanced fatty acid composition and that the high omega-6 content can create an environment that favours inflammation and hepatic damage (4). Consequently, lipid emulsions that contain fish oil, a source of EPA and

DHA, blended with soybean and other oils have been developed (2-4). Clearly by containing less soybean oils these blends contain less linoleic acid. However, the inclusion of EPA and DHA is seen as important because these long chain omega-3 PUFAs have significant bioactivity including reducing inflammation (2-5). In addition, an emulsion based solely on fish oil has been developed (2). The study of Noureddine et al. (1) compared emulsions based solely on soybean or fish oils. Fish oil containing lipid emulsions have been used in surgical and critically ill patients where they are reported to decrease infections and length of intensive care unit and hospital stay and, in some studies, to decrease mortality (6,7,8). They have also been used in adult patients receiving long-term intravenous nutrition support at home (9) and in various pediatric settings (10). In the latter, inclusion of fish has been shown to reduce the risk of, and to reverse existing, intestinal failure associated liver disease (11).

In all settings described above, the central mechanism of action of EPA and DHA is the control of inflammation, which occurs through various inter-related mechanisms (5). It has been recognised for decades that EPA and DHA decrease production of pro-inflammatory eicosanoid mediators from the omega-6 PUFA arachidonic acid and that EPA gives rise to analogous, generally weakly bioactive, eicosanoids. More recently, it has been discovered that EPA and DHA are substrates for the production of highly bioactive SPMs that, in addition to anti-inflammatory actions, actively resolve inflammation (12,13). SPMs include E-series resolvins produced from EPA and D-series resolvins, protectins and maresins produced from DHA. It is emerging that SPMs may be responsible for many of the biological actions ascribed to EPA and DHA.

Noureddine et al. (1) infused C57BL/6 mice with commercial soybean oil or fish oil based lipid emulsions for 7 days. Lipid mediators, including SPMs, in the liver and spleen were measured by UHPLC tandem MS. Data were subjected to principal component analysis (PCA). For both tissues, the lipid mediator profiles in mice receiving soybean oil or fish oil were clearly separated. The separation was mainly due to the presence of EPA- and DHA-derived lipid mediators including SPMs and also hydroxy-EPA and hydroxy-DHA metabolites that are the intermediates in the biosynthesis of SPMs from EPA and DHA. While the tissues from mice that received the soybean oil lipid emulsion contained higher concentrations of lipid mediators from linoleic, α-linolenic, dihomo-γ-linolenic and arachidonic acids (including several pro-inflammatory eicosanoids), those from mice that received the fish oil lipid emulsion contained higher concentrations of several hydroxy-EPAs and -DHAs, some EPA-derived eicosanoids, EPA- and DHA-containing endocannabinoids, and resolvins D1, D2, D4 and D5, maresins 1

and 2, protectin D1 and protectin DX, all produced from DHA. In the liver, 36 out of 51 detectable lipid mediators were different between mice receiving the two lipid emulsions; for the spleen this figure was 46 out of 51 detectable mediators. The spleen contained higher concentrations of lipid mediators than the liver, consistent with the spleen being a more immunologically active tissue.

The second part of the study of Noureddine et al. (1) involved incubation of human blood-derived T cells and macrophages differentiated from blood-derived monocytes for 48 hours with the two different lipid emulsions used at three different concentrations. Incubation of both cell types with the fish oil-based emulsion resulted in a much higher cell content of EPA and DHA and a decrease in content of omega-6 PUFAs. The incorporation of omega-3 PUFAs reported is far in excess of what is seen for rat leucocytes following infusion of this same lipid emulsion over 3 days (14). This may reflect that the concentration of lipid emulsion achieved in vitro may be much higher than that achieved in the rats and the constant exposure of the cells to the lipid emulsion in vitro. Interestingly incubation with the soybean oil-based emulsion had little impact on cell fatty acid composition. To study the macrophages, the 48 hour exposure to lipid emulsion was followed by 48 hour exposure to bacterial lipopolysaccharide and then lipid mediators were measured intracellularly. Once again, the PCA showed separation according to lipid emulsion used and once again the fish oil-based lipid emulsion resulted in higher concentrations of several hydroxy-EPAs and DHAs, some EPA-derived eicosanoids, EPA-ethanolamide and resolvin D5. There was also a lower concentration of the potent pro-inflammatory leukotriene B_4 produced from arachidonic acid. There are two features of this experiment that are worth remarking on. The first is that the culture time with lipopolysaccharide of 48 hours seems rather long since lipid mediators are fairly rapidly synthesised upon cellular stimulation. As a comparison, others have stimulated human monocyte-derived macrophages for 1 hr with apoptotic granulocytes (15), for 90 or 180 min with bacteria (16) or for 1 hr with lipopolysaccharide (16) to measure lipid mediator generation. The second feature is that Noureddine et al. (1) measure intracellular mediators, whereas most other studies measure mediators in the culture medium (i.e. extracellular) or in the combination of the cells and the culture medium (i.e. intra plus extracellular). It would seem that extracellular concentrations are the most relevant since the mediators act via cell surface G-protein coupled receptors upon release.

To study $CD4^+$ ("helper") T cells, Noureddine et al. (1) incubated the cells with the lipid emulsion and simultaneously with T cell activator for 48 hr. Again, the PCA showed separation according to lipid emulsion used. However, the mediators mainly contributing to

this separation were different from what was observed for the macrophages: these were mainly various hydroxy-EPAs, although some hydroxy-DHAs and EPA-ethanolamide were also higher in the fish oil lipid emulsion treated cells.

The research of Noureddine et al. (1) has strengths and weaknesses. Strengths include its scientific novelty, the measurement of lipid mediators in tissues, and the high quality of the lipid mediator analysis. However, both the in vivo and in vitro studies have limitations with regard to modelling the human clinical situation in which patients will receive intravenous lipid emulsions. This approach is used in critical illness, following major trauma or major surgery, or in patients with severe acute or chronic intestinal dysfunction. The mice studied by Noureddine et al. (1) were healthy and did not undergo any challenge that might mimic the human clinical settings to which the research can be applied. Furthermore, in most clinical settings, the insult (e.g. trauma, intestinal failure) would occur prior to the administration of the lipid emulsion. However, Noureddine et al. (1) exposed macrophages to lipid emulsion for 48 hours prior to the challenge of lipopolysaccharide and they exposed CD4+ T cells to lipid emulsions at the same time as the immune activation. The concentrations of omega-3 PUFAs achieved in the cells in the in vitro experiments (up to 40% of fatty acids for macrophages and up to 50% of fatty acids for $CD4^+$ T cells) are very high and would be unlikely to be achieved in any patient setting. Barros et al. (14) reported total omega-3 PUFAs in rat leukocytes reached 5% following 3 hr infusion of fish oil-based lipid emulsion each day for 3 consecutive days. One approach to optimizing the amount of lipid emulsion to be used in vitro experiments would be to identify the omega-3 PUFA content of leukocytes following infusion of the fish oil-based lipid emulsion in the mice studied by Noureddine et al. (1) and to establish the concentration required to achieve that content in vitro. This would allow for a clearer link between the in vivo and in vitro studies.

The research described by Noureddine et al. (1) is a novel approach to enhance understanding of the influence of fish oil containing lipid emulsions and to explain the biological and clinical effects that have been observed in various patient groups (2-4,6-11). In both mice and isolated cells in culture, exposure to soybean oil- or fish oil-based lipid emulsions resulted in markedly different profiles of lipid mediators with fish oil favoring the appearance of the hydroxy precursors of SPMs and of the SPMs themselves. In parallel with this was reduced appearance of pro-inflammatory eicosanoids from omega-6 PUFAs. Poor patient outcome, for example following major surgery, in critical illness, and in those requiring long-term intravenous nutrition support, is considered to involve an excessive inflammatory response. Lipid emulsions that include fish oil have been reported to improve patient outcomes

(2,4,6-11). One mechanism might be the promotion of an inflammation-resolving environment as a result of production of hydroxy-EPAs and -DHAs which have biological activity and of SPMs. Hence the findings of Noureddine et al. (1) make a useful contribution to this field. There are reports of alteration in eicosanoid profiles in patients undergoing major surgery with post-surgical intravenous administration of a lipid emulsion containing fish oil (17,18). Measuring a broad range of bioactive lipid mediators, including SPMs, in the bloodstream of patients receiving different lipid emulsions and relating these to biomarkers (e.g. of inflammation) and to clinical outcome should be the next step.

References

1. Nouredine N, Hartling I, Wawrzyniak P, Srikanthan P, Lou P-H, Luccinetti E, Kramer SD, Rogler G, Zaugg M, Hersberger M. Lipid emulsion rich in n-3 polyunsaturated fatty acids elicits a pro-resolution lipid mediator profile in mouse tissues and in human cells. American Journal of Clinical Nutrition 2022;in press.
2. Calder PC, Adolph M, Deutz NE, Grau T, Innes JK, Klek S, Lev S, Mayer K, Michael-Titus AT, Pradelli L, Puder M, Vlaardingerbroek H, Singer P. Lipids in the intensive care unit: Recommendations from the ESPEN Expert Group. Clinical Nutrition 2018;37:1-18.
3. Calder PC, Waitzberg DL, Klek S, Martindale RG. Lipids in parenteral nutrition: biological aspects. JPEN Journal of Parenteral and Enteral Nutrition 2020;44 Suppl 1:S21-S27.
4. Calder PC. Lipids for intravenous nutrition in hospitalised adult patients: a multiple choice of options. Proceedings of the Nutrition Society 2013;72:263-276.
5. Calder PC. n-3 PUFA and inflammation: from membrane to nucleus and from bench to bedside. Proceedings of the Nutrition Society 2020;79:404-416.
6. Calder PC. Intravenous lipid emulsions to deliver bioactive omega-3 fatty acids for improved patient outcomes. Marine Drugs 2019;17:274.
7. Pradelli L, Mayer K, Klek S, Omar Alsaleh AJ, Clark RAC, Rosenthal MD, Heller AR, Muscaritoli M. ω-3 Fatty-acid enriched parenteral nutrition in hospitalized patients: systematic review with meta-analysis and trial sequential analysis. JPEN Journal of Parenteral and Enteral Nutrition 2020;44:44-57.
8. Martindale RG, Berlana D, Boullata JI, Cai W, Calder PC, Deshpande GH, Evans D, Garcia-de-Lorenzo A, Goulet OJ, Li A, Mayer K, Mundi MS, Muscaritoli M, Pradelli L, Rosenthal M, Seo JM, Waitzberg DL, Klek S. Summary of Proceedings and Expert

Consensus Statements From the International Summit "Lipids in Parenteral Nutrition". JPEN Journal of Parenteral and Enteral Nutrition 2020;44 Suppl 1:S7-S20.

9. Ahmed S, Innes JK, Calder PC. Influence of different intravenous lipid emulsions on fatty acid status and laboratory and clinical outcomes in adult patients receiving home parenteral nutrition: A systematic review. Clinical Nutrition 2021;40:1115-1122.
10. Edward RR, Innes JK, Marino LV, Calder PC. Influence of different intravenous lipid emulsions on growth, development and laboratory and clinical outcomes in hospitalised paediatric patients: A systematic review. Clinical Nutrition 2018;37:765-783.
11. Nandivada P, Fell GL, Gura KM, Puder M. Lipid emulsions in the treatment and prevention of parenteral nutrition-associated liver disease in infants and children. American Journal of Clinical Nutrition 2016;103:629S-634S.
12. Chiang N, Serhan CN. Specialized pro-resolving mediator network: an update on production and actions. Essays in Biochemistry 2020;64:443-462.
13. Dyall SC, Balas L, Bazan NG, Brenna JT, Chiang N, da Costa Souza F, Dalli J, Durand T, Galano JM, Lein PJ, Serhan CN, Taha AY. Polyunsaturated fatty acids and fatty acid-derived lipid mediators: Recent advances in the understanding of their biosynthesis, structures, and functions. Progress in Lipid Research 2022;86:101165.
14. Barros KV, Carvalho PO, Cassulino AP, Andrade I, West AL, Miles EA, Calder PC, Silveira VL. Fatty acids in plasma, white and red blood cells, and tissues after oral or intravenous administration of fish oil in rats. Clinical Nutrition 2013;32:993-998.
15. Dalli J, Serhan CN. Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood 2012;120:e60-72.
16. Werz O, Gerstmeier J, Libreros S, De la Rosa X, Werner M, Norris PC, Chiang N, Serhan CN. Human macrophages differentially produce specific resolvin or leukotriene signals that depend on bacterial pathogenicity. Nature Communications 2018;9:59.
17. Wachtler P, König W, Senkal M, Kemen M, Köller M. Influence of a total parenteral nutrition enriched with omega-3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. Journal of Trauma 1997;42:191-198.
18. Grimm H, Mertes N, Goeters C, Schlotzer E, Mayer K, Grimminger F, Fürst P. Improved fatty acid and leukotriene pattern with a novel lipid emulsion in surgical patients. European Journal of Nutrition 2006;45:55-60.