Partitioning of α-Linolenic acid metabolism towards 20:3n-3 synthesis rather than 18- carbon oxylipin production alters with age, which is consistent with induction of a more inflammatory phenotype in older individuals

J. von Gerichten¹, N.A. Irvine², A.L. West², K. Lillycrop³, E.A. Miles², P.C. Calder²,4, G.C. Burdge² and B.A. Fielding¹
¹Department of Nutritional Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK,
²School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, Hampshire, UK,
³Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton, Hampshire, UK and
⁴NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, Hampshire, UK

The essential fatty acid (EFA) α-linolenic acid (ALA, 18:3n-3) can be metabolised into longer chain n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid and docosahexaenoic acid. ALA can also be oxidised into immunomodulatory lipid mediators, such as 18-carbon oxylipins, including hydroxyoctadecatrienoic acids (HOTrEs). Because HOTrE synthesis is impaired in inflammatory disease, we hypothesised that partitioning of ALA towards HOTrE synthesis is reduced in T cells from older individuals as a possible mechanism in age-related immune dysregulation known as immunosenescence(1). To test this, peripheral blood CD3+ T cells from healthy younger adult volunteers (18–30 years; n=10) and older adult volunteers (58–74 years; n=6) were cultured for 48 h, with or without concanavalin A (10 μg/mL) in 10% (v/v) pooled donor plasma, in media with a 5:1 linoleic acid (LA, 18:2 n-6) to ALA ratio. Total ALA included [13C] ALA, to trace the relative partitioning of ALA. ALA metabolites were detected either by gas chromatography-mass spectrometry for cellular PUFA or by LC-MS/MS for oxylipins in cell culture supernatant (2). Metabolite to ALA ratios were calculated and for the primary PUFA synthesised from ALA in T cells, eicosatrienoic acid ([13C]20:3n-3), and the most abundantly oxidised metabolite of ALA, [13C]9-HOTrE. Multiple t-test (unpaired, two-tailed) with Holm-Sidak correction was performed for statistical analysis on log transformed data (GraphPad Prism 8.4.3). Results for stimulated and unstimulated cells were similar; only results for stimulated cells are reported here. Oxylipin synthesis, measured as [13C]9-HOTrE / ALA was lower in cells from older adults compared to younger adults (median (range), 2.7 (1.1–4.0) vs 5.4 (2.1–9.6), P = 0.01). We then compared the relative partitioning of ALA into the alternative metabolic pathway and found that [13C]9-HOTrE / ALA was lower in cells from older adults compared to younger adults (median (range), 2.7 (1.1–4.0) vs 0.02 (0.004–0.036), P < 0.001), in younger as well as in older adults (2.7 (1.1–4.0) vs 0.04 (0.02–0.08), P < 0.001).

In conclusion, using a stable isotope tracer, we found that mitogen-stimulated T cells took up ALA added to the culture medium and preferentially used it for the constitutive production of 9-HOTrE, rather than synthesis of longer chain PUFAs in both younger and older adults. However, partitioning altered with age, towards 20:3n-3 synthesis rather than 18-carbon oxylipin production, which is consistent with induction of a more inflammatory phenotype in older individuals. This has implications for understanding the role of essential fatty acids in immunosenescence.

Acknowledgments
This research was supported by grants from the Biological Sciences and Biotechnology Research Council (grant numbers BB/S00548X/1, BB/R00028X/1 and BB/S005358/1).
The authors wish to acknowledge Dr Debra Skene and the Metabolomics Core Facility, School of Bioscience and Medicine, University of Surrey, for providing support and resources for the LC-MS analysis of oxylipins.

References