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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

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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 [^{13}C] 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 ([^{13}C]20:3n-3), and the most abundantly oxidised metabolite of ALA, [^{13}C]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 [^{13}C]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 [^{13}C]9-HOTrE / ALA was markedly higher than [^{13}C] 20:3n-3 / ALA (5.4 (2.1–9.6) 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.

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