# Arachidonic Acid and Docosahexaenoic Acid are Metabolized Differently by Microglia Cells

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Microglia cells are the main source of oxidized lipid species in the brain, some of them widely known by their role in the inflammatory response. Such species received the label of “inflammatory lipid mediators”, and can be divided in pro-inflammatory or pro-resolution according to their lipid precursor: arachidonic acid (ARA, n-6) or docosahexaenoic acid (DHA, n-3), respectively. Given that lipid metabolism significantly influences microglia activation phenotype and fatty acid availability is a limiting factor for the production of lipid mediators, we aimed to investigate whether ARA and DHA are processed differently by microglia cells and how this processing relates to their role in inflammation. For this study, we supplemented BV2 microglia cells with high (100 uM) and low (20 uM) concentration of ARA or DHA, followed by mass spectrometry analysis using a lipidomics approach. Our findings revealed that, when supplemented in high concentrations, DHA significantly accumulates in the cell (6.3 fold) where is preferably incorporated into phosphatidylethanolamine (PE), plasmenyl-phosphatidylethanolamine (pPE) and cholesterol esters (CE). DHA supplementation also increased total glycerolipids inside the cell. On the other hand, ARA accumulates in a smaller proportion compared to DHA (1.6 fold), and even though its incorporation into PE and pPE is similar to DHA, ARA supplementation actually decreases CE and glycerolipids levels. These observations suggest that microglia BV2 preferably stores DHA compared to ARA, which may be rapidly metabolized or taken up from the media less effectively. To further investigate this, we search for the oxidized products of both fatty acids using a targeted mass spectrometry method specifically designed for oxidized species (oxilipidomics). Our results showed that DHA supplementation increased the production of monohydroperoxides derived from DHA (4-, 8-, 10-, 13-, 15-, 16-, 17- and 20-HDoHe), while ARA supplementation decreased monohydroperoxides derived from ARA (5-, 11-HETE) and increased the level of 11(12)-EET, an epoxide produced by the reaction of ARA with the CYP450 enzyme. Overall, our findings suggest that ARA and DHA are metabolized differently by microglia BV2 cells, DHA being preferably stored in lipid droplets while ARA is rapidly metabolized, although not necessarily as precursor to lipid mediators under non-inflammatory conditions.

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