

Supplementary material 1

Bligh-Dyer lipid extraction method

The methodology was adapted from Bligh-Dyer method [18]. Aliquots of 100 μL plasma were transferred in Eppendorf tubes and mixed with 10 μL of internal standard mixture containing 10 $\mu\text{g}/\text{mL}$ DHA-d5, ARA-d8 and or EPA-d5, and 10 $\mu\text{g}/\text{mL}$ LA-d4 and ALA-d1. 750 μL of chloroform/methanol mixture (1:2, v/v). The tubes were vortexed and maintained at -20°C for 10 min, then centrifuged at 14,000 g at 4°C for 5 min. The supernatant was transferred into a clean tube. 250 μL of chloroform and 250 μL water were added. The tubes were placed on a rotary mixer for 5 min and then centrifuged for 5 min at 1000 g.

The bottom layer was collected, transferred in a glass tubes and dried under nitrogen flow. 1 mL of 80% methanol was added, and the tubes were thoroughly mixed. Of this, a volume of 100 μL was transferred into an autosampler vial for free fatty acids analysis. The remaining aliquot was subjected to alkaline hydrolysis as described in section 3.3.2.