Erythrocyte omega-3 status of ADNI-3 participants

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Introduction

The aim of the study was to assess fatty acids profile in the red blood cells of ADNI-3 participants, with special regards to long chain polyunsaturated fatty acids (PUFA), i.e. eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3).

Summary

The study, based on samples of ADNI-3 participants, consist in the analysis of fatty acids composition of the red blood cells to assess the omega-3 status (%EPA+%DHA) of the volunteers. The analysis was performed from March to June 2021 with 849 individual samples. After weighing a sample of red blood cells, total lipids were extracted, converted to fatty acid methyl esters (FAME) and analyzed by GC/MS (agilent 7890N / 5975C system).

Methodology

Lipids were extracted twice from red blood cells (475-525 mg) samples with a mixture of hexane/isopropanol (3:2 v/v), after acidification with 1 mL HCl 3 M [1], containing 5 ppm BHT. Margaric acid (C17:0) was added as internal standard.

Total lipid extracts were saponified with 1 mL of 0.5 M NaOH in methanol for 30 min at 70 °C and methylated with 1 mL of BF_3 (12% w/v in methanol) for 15 min at 70°C. Fatty acid methyl esters (FAME) were extracted twice with pentane. Solvent was removed under nitrogen and FAME redissolved in 200µL of hexane.

Analysis were performed using an Agilent Technologies 7890N gas chromatograph (Bios Analytic, Toulouse, France) fitted with a split injector (10:1) at 250 °C (injection volume 1 µL)

and a bonded silica capillary column (BPX 70, 60 m long 250 µm inner diameter and 0.25 µm film thickness; SGE, Villeneuve-St Georges, France). Helium was used as a carrier gas (constant flow: 1.8 mL/min, 36 cm/sec).

The column temperature program started at 170 °C, increased by 4 °C/min to 250 °C and held at 250 °C for 2 min. Transfer line was at 270°C. Mass spectra were obtained with an Agilent 5975C spectrometer used in electron impact mode (EI) with 70 eV energy, source temperature was set at 150°C and quadrupole at 230°C. Acquisition was performed in the full scan mode ranging from 50 to 500 amu (3 scan/s).

Identification of FAME was based on retention times obtained for FAME prepared from fatty acid standards and confirmed by comparison of their MS spectra with those of the NIST bank (V.2.2, 2014).

Quantification was achieved by determining the area under the peaks with Mass Hunter software (version B.07.00 SP2, 2015, Agilent). Results are expressed as % of total identified fatty acids. DHA concentration was calculated using the internal standard and expressed as µg of DHA/g red blood cells.

References

1. Comparative effects of dietary n-3 docosapentaenoic acid (DPA), DHA and EPA on plasma lipid parameters, oxidative status and fatty acid tissue composition Gaetan Drouin, Daniel Catheline, Etienne Guillocheau, Pierre Gueret, Charlotte Baudry, Pascale Le Ruyet, Vincent Rioux, Philippe Legrand, *J. Nutr. Bioch.*, 2019, 63, 186-196.

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