UNIVERSITY OF WATERLOO
FACULTY OF APPLIED HEALTH SCIENCES
Department of Kinesiology

Quantitative Macrolipidomics of Human Whole Blood for the Discovery of Novel Biomarkers of Omega-3 Polyunsaturated Fatty Acid Intake

Juan J. Aristizabal-Henao[1], Ningombam Sanjib Meitei[2], Anja Pia Biltoft-Jensen[3], & Ken D. Stark[1]

[1]Laboratory of Nutritional Lipidomics, Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada

[2]PREMIER Biosoft, Palo Alto, CA, USA

[3]Technical University of Denmark, Lyngby, Denmark

Scan For More Information

NSERC CRSNG

Overview

- **Purpose**: To identify and quantitate highly-abundant complex lipids in human whole blood (i.e. the *macrolipidome*) that are correlated with the dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).
- **Methods**: Lipid extracts were obtained from whole blood samples (n=120) as part of a standardized dietary assessment project of the Danish National Food Institute. Samples were analyzed using reversed-phase UHPLC and a polarity-switching MS/MS method. Lipid species were quantitated using a mix of 14 deuterium-labelled internal standards.
- **Results**: Omega-3 polyunsaturated fatty acid (PUFA) lipid species in blood were more strongly correlated with intakes of DHA, followed by EPA intakes and total omega-3 PUFA intakes.

Introduction

The relationship between diet and blood levels of omega-3 PUFA has been studied extensively. Most of this research has been focused on overall fatty acid metabolism in total lipids or lipid fractions of plasma/serum and erythrocytes using gas chromatography-flame ionization detection (GC-FID). Fatty acid compositional data derived from GC-FID-based analyses rely on the hydrolysis and methylation or direct transesterification of fatty acyl chains on complex lipids to generate fatty acid methyl esters. This results the inability to characterize complex lipid species in their native state and structural information that could be of physiological relevance is lost. Ultra-high performance liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS)-based analyses have the potential to characterize complex lipids as they exist in their natural states

Methods

Sample Collection and Dietary Assessment

Human blood samples (n = 120) were collected as part of the KOMET standardized dietary assessment project of the Danish National Food Institute. Dietary intakes for all participants were assessed using 7-day food records for macronutrients, fatty acid classes, and some individual fatty acids through the Danish Food Composition Database (https://frida.fooddata.dk). Whole blood samples were shipped to Waterloo, ON, Canada on dry ice, and were kept at -80ºC until sample preparation.

Lipid Extraction and Analysis by UHPLC-MS/MS

Lipid extracts were obtained from 50µL aliquots of whole blood using 3mL of 2:1 chloroform/methanol (v/v) which delivered known amounts of deuterium-labelled internal standards for the major lipid classes (Splash Lipidomix, Avanti Polar Lipids, Alabaster, AL, USA). Lipid extracts were dried under N_2 gas and reconstituted in 100µL of 65:35:5 acetonitrile/isopropanol/water (v/v/v) +0.1% formic acid. Samples were then vortexed briefly and stored in vials at 4ºC until analysis by UHPLC-MS/MS.

Lipidomic analyses were completed using an Acquity UPLC system coupled to a Synapt G2S*i* QToF mass spectrometer (Waters, Milford, MA, USA). A binary multi-step gradient was used with a Waters Acquity UPLC CSH column, 1.7µm x 2.1mm x 150mm. The mass spectrometer was operated in the negative ion mode (-2.25kV) from 0min to 27min, and in the positive ion mode (+2.25kV) from 27min to 47min (**Figure 1**). The scan range was *m/z* 100 to 1200. Tandem mass spectrometry was performed using a top-5 data-dependent acquisition method.

Statistical Analyses

Lipid identifications were made using SimLipid (version 6.02, Palo Alto, CA, USA), and peak areas were integrated using Progenesis QI (version 2.3, Newcastle upon Tyne, UK). Lipid abundances were normalized using the internal standard belonging to the same lipid class as the analyte of interest. Two-tailed bivariate correlations were performed between dietary intake data and lipid concentrations using SPSS for Windows (release 11.5.1, Chicago, IL, USA), statistical significance was inferred at $p < 0.05$.

Results

Figure 1. Total Ion Chromatogram and Approximate Elution Times of the Major Lipid Classes

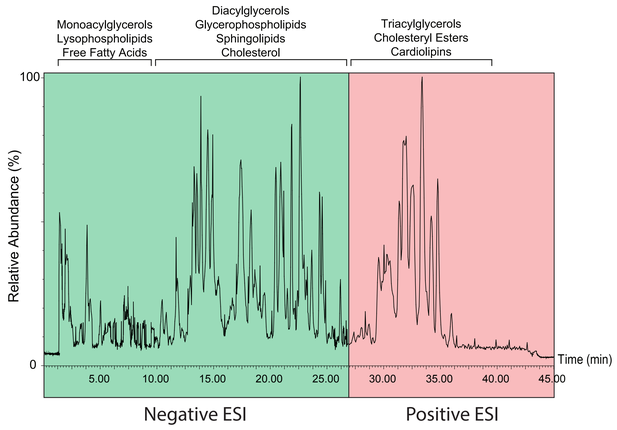
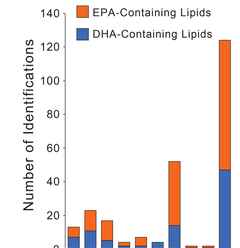


Figure 2. Number of EPA- or DHA-Containing Complex Lipids

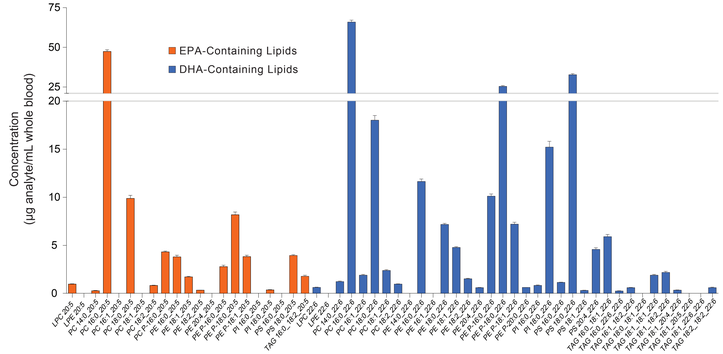


PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; PG, phosphatidylglycerol; TAG, triacylglycerol; CE, cholesteryl ester; FFA, free fatty acid.

Table 1. Participant Summary

Characteristic	N = 120 (52 M, 68 F)
Age (years)	39 ± 12
Body Mass Index (kg/m²)	24.17 ± 3.23
Dietary Intake	
Total Energy (kJ)	9448.99 ± 2456.20
Protein (g/d)	83.42 ± 24.05
Carbohydrate (g/d)	237.57 ± 69.67
Fat (g/d)	89.83 ± 27.11
Saturated Fat (g/d)	33.57 ± 11.49
C 12:0	1.30 ± 0.75
C 14:0	3.25 ± 1.40
C 16:0	17.97 ± 5.91
C 18:0	7.55 ± 2.82
Monounsaturated Fat (g/d)	31.18 ± 10.57
C 16:1n-7	1.52 ± 0.66
C 18:1n-9	29.66 ± 10.10
Polyunsaturated Fat (g/d)	13.49 ± 4.64
Omega-6 Fatty Acids (g/d)	11.15 ± 3.85
C 18:2n-6	11.09 ± 3.84
C 20:4n-6	0.12 ± 0.07
Omega-3 Fatty Acids (g/d)	2.06 ± 1.14
C 18:3n-3	1.73 ± 1.04
C 20:5n-3	0.10 ± 0.11
C 22:5n-3	0.04 ± 0.04
C 22:6n-3	0.16 ± 0.17

Figure 3. Concentrations of the Major EPA- or DHA-Containing Lipids in Whole Blood

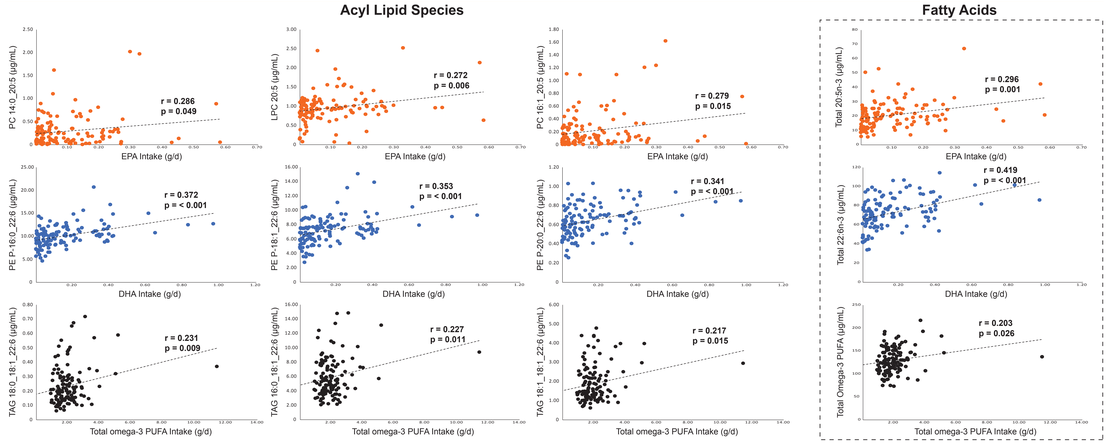


LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TAG, triacylglycerol.

Conclusions

- Mean intakes of EPA, DHA and total omega-3 PUFA were approximately 0.10, 0.16 and 2.06 grams/day, respectively.
- There were 124 EPA- or DHA-containing species identified in whole blood across several lipid classes, including phospholipids, triacylglycerols, free fatty acids and cholesteryl esters.
- The omega-3 lipids with the highest abundances were PC 16:0_20:5, PC 16:0_22:6, PE P-18:0_22:6 and PS 18:0_22:6.
- Omega-3 lipid species in blood (lipidomics) were correlated more strongly with DHA intakes, followed by EPA intakes and total omega-3 PUFA intakes. The lipidomic-intake correlations were similar to the fatty acid-intake correlations.
- Future work should focus on examining the relationship between omega-3 lipid species in blood and dietary patterns such as sporadic or regular consumption of different types of omega-3 PUFA.

Figure 4. Correlations Between the Intakes of Omega-3 PUFA and Concentrations of Omega-3 Fatty Acids and Lipids in Blood



The complex lipids with the strongest correlations (Pearson r-values) to dietary EPA (orange), DHA (blue), and total omega-3 PUFA (black) are shown, as well as plots of total EPA concentration vs. EPA intake, total DHA concentration vs. DHA intake, and total omega-3 PUFA concentrations vs. omega-3 PUFA intake (within dotted box). All correlations were statistically-significant with $p < 0.05$. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TAG, triacylglycerol.