A quick efficient method to monitor omega-3 fatty acid intake in nutritional follow-up

B. Babiy [2], S. Recuero [2], M. Cofán [1,3], C. Vázquez [1,5], A. Alcázar [2], R. Busto[1,2], A. Sureda [1,4], J.M. Lou [1,6], O. Pastor [1,2]

1) CIBEROBN, ISCIII, Spain. 2) Servicio de Bioquímica-Investigación y Bioquímica Clínica, Hospital Universitario Ramón y Cajal (IRyCIS), Madrid. 3) Servicio de Endocrinología y Nutrición, Hospital Clínic de Barcelona, Instituto de Investigaciones Biomédicas August Pi Sunyer (IDIBAPS). 4) Grupo de Nutrición Comunitaria y Estrés Oxidativo, Dpto. Biología y Ciencias de la Salud, Universidad de las Islas Baleares. 5) Servicio de Endocrinología y Nutrición, Hospital Universitario Fundación Jiménez Díaz, Madrid. 6) Dpto. Farmacología y Fisiología, Universidad de Zaragoza.

email: oscar.pastor@salud.madrid.org

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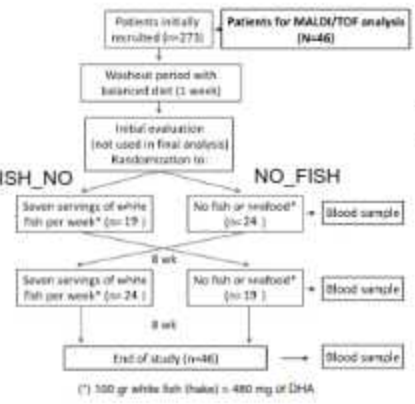
INTRODUCTION & AIMS

Omega-3 fatty acids are essential nutrients with potentially beneficial roles in the prevention of inflammatory and cardiovascular diseases. Therefore, it is important to provide reliable and productive analytical methodologies for the measurement of fatty acids in the population. The goal of this study is to develop a fast analysis to monitor the levels of omega-3 fatty acids which can be applied in large population studies and for everyday nutritional follow-up. We checked the performance of a lipidomics approach using MALDI-TOF/MS technology, which allows the measurement of phosphatidylcholine species (LPC and PC) containing omega-3 (**Figure 1 & 2**), in comparison to a Fast GC-MS approach (**Figure 3**). Both methodologies were employed to follow the omega-3 enrichment in plasma samples from two nutritional intervention studies: WISH-CARE (White Fish for Cardiovascular Risk Factors in Patients with Metabolic Syndrome) (**Figure 5**) and WAHA (Walnuts and Healthy Aging Study) (**Figures 4 & 6**).

PATIENTS & METHODS

- ✓ We selected 46 plasma samples from the WISH-CARE and 113 patients from the WAHA study.
- ✓ A fast measurements of ω-3 PUFAs in plasma were developed in a MALDI-TOF/MS platform (**Figure 1**). ALA (α-linolenic) species in MALDI were obtained by the sum of phospholipids LPC 18:3, PC 34:3, PC 36:3, DHA (docosahexaenoic) species were obtained by and LPC 22:6, PC 38:6 and PC 40:6.
- ✓ Samples were also analysed by GC-MS using a fast extraction and derivatization protocol.

Study Design of WISH-CARE [1]



Study Design of WAHA [2]

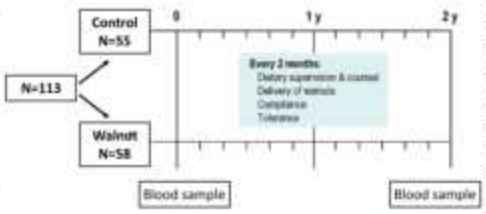
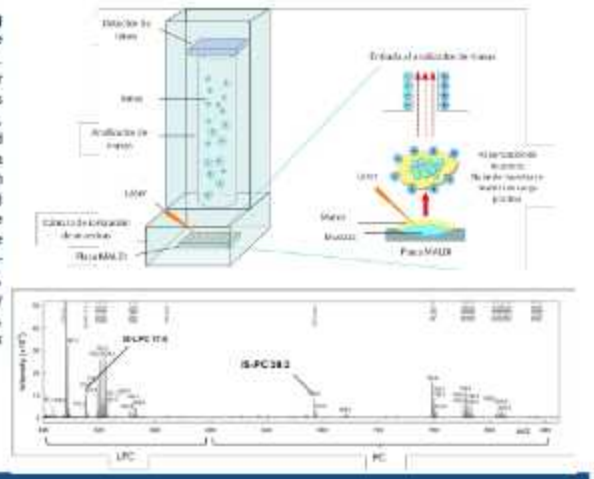


Figure 1. Fast PC and LPC lipidomics using MALDI-TOF/MS. A) Plasma samples were mixed with matrix and spotted on a target plate. Ionization of phospholipids are induced by laser and ions are directed to a Time of Flight mass spectrometer. The spectrogram (**Figure 1**), allows the identification and quantitation of lipid species. **B)** Representative spectrogram of a plasma sample. Plasma (10 μL) was mixed with 90 μL of ACN (containing internal standards) and centrifuged. 10 μL of the upper phase were mixed with 90 μL of IPA/ACN (60/40). The solution was blended 1:1 (v/v) with matrix, 9-aminoacridine solution (10 mg/mL in IPA/ACN, 60/40). The matrix-sample mixture was finally spotted (0.65 μL) on a MALDI 384 well plate, and mass spectra acquired on a Bruker Autoflex III MALDI-TOF spectrometer.

MALDI-TOF/MS analysis of phospholipids [3]



1. Vázquez C. Nutr Metab Cardiovasc Dis. 2014 Mar;24(3):328-35. PMID:24462043.
2. Rajaram S. Front Aging Neurosci. 2017 Jan 10;8:333. PMID:28119602.
3. Pastor O. J Nutr Biochem. 2019 Jan;63:197-205. PMID:30414540.

RESULTS

Performance of MALDI-TOF/MS for plasma

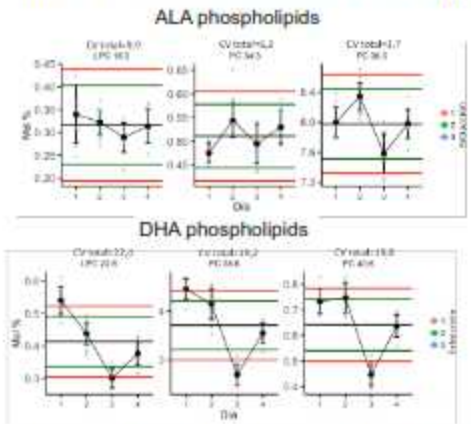


Figure 2. Reproducibilty of MALDI-TOF measurement of plasma ALA and DHA. A pool of plasma was analysed four times during four days. Green lines and red lines mark 2sd and 3sd deviations from the average values.

Performance of Fast GC-MS method

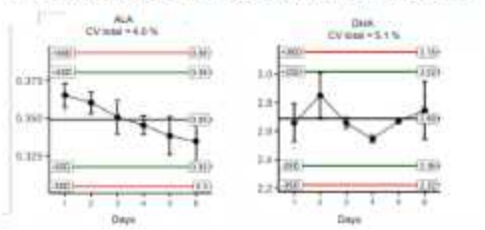


Figure 3. Reproducibilty of fast GC-MS measurement of plasma ALA and DHA. A pool of plasma was analysed four times during 6 days. Green lines and red lines mark 2sd and 3sd deviations from the average values.

MALDI-TOF/MS in WAHA patients

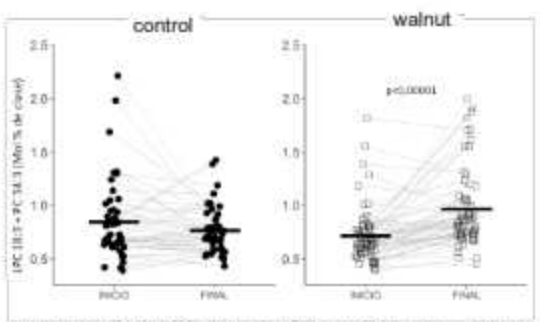


Figure 4 . MALDI-TOF/MS analysis of DHA species in plasma form the WAHA study. ALA was measured as the combined sum of LPC 18:3 + PC 34:3 and PC 36:3 species. The analysis demonstrated the response to supplementation in the walnut group.

MALDI-TOF/MS in WISH-CARE patients

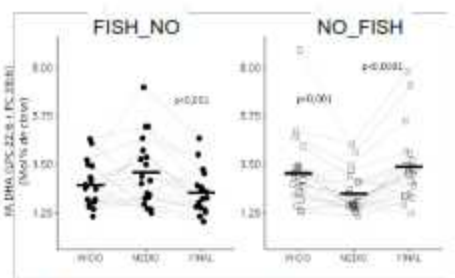


Figure 5. MALDI-TOF/MS analysis of DHA species in plasma form the WHIS-CARE study. DHA was measured as the combined sum of LPC 22:6 + PC 38:6 + PC 40:6). The analysis demonstrated the response to white fish supplementation in both groups of patients.

Fast GC-MS in WAHA patients

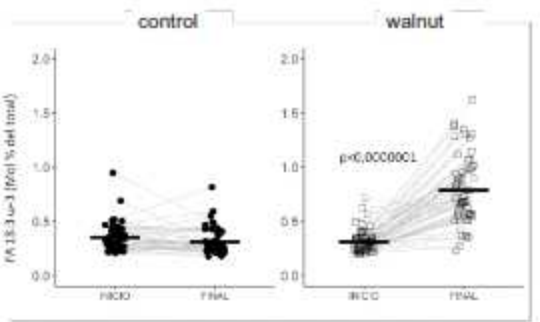


Figure 6. GC-MS analysis of ALA in plasma in patients from the WAHA study. The analysis demonstrated the response to walnut supplementation, with much better reproducibility than MALDI-TOF/MS. ALA = FA 18:3 w-3.

CONCLUSIONS

- ✓ The quantification of ALA and DHA in phospholipids by MALDI-TOF/MS is rapid and simple but the between-day reproducibity of the method is insufficient.
- ✓ On the contrary, fast GC-MS analysis have an excellent global reproducibility for ALA and DHA (and other fatty acid species). Besides, the method increases the productivity up to 150 samples/week. The method is suitable for nutritional follow-up in large population studies.

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