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Translating plasma and whole blood fatty acid compositional data into the sum of eicosapentaenoic and docosahexaenoic acid in erythrocytes

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ABSTRACT

Specific blood levels of eicosapentaenoic plus docosahexaenoic acid (EPA+DHA, wt% of total) in erythrocytes or "the omega-3 index" have been recommended for cardio-protection, but fatty acids are often measured in different blood fractions. The ability to estimate the % of EPA+DHA in erythrocytes from the fatty acid composition of other blood fractions would enable clinical assessments of omega-3 status when erythrocyte fractions are not available and increase the ability to compare blood levels of omega-3 fatty acids across clinical studies. The fatty acid composition of baseline plasma, erythrocytes and whole blood samples from participants ($n=1104$) in a prospective, multicenter study examining acute coronary syndrome were determined. The ability to predict the % of EPA+DHA in erythrocytes from other blood fractions were examined using bivariate and multiple linear regression modelling. Concordance analysis was also used to compare the actual erythrocytes EPA+DHA values to values estimated from other blood fractions. EPA+DHA in erythrocytes was significantly ($p<0.001$) correlated EPA+DHA in plasma ($r^2=0.54$) and whole blood ($r^2=0.79$). Using multiple linear regression to predict EPA+DHA in erythrocytes resulted in stronger coefficients of determination in both plasma ($R^2=0.70$) and whole blood ($R^2=0.84$). Concordance analyses indicated agreement between actual and estimated EPA+DHA in erythrocytes, although estimating from plasma fatty acids appears to require translation by categorization rather than by translation as continuous data. This study shows that the fatty acid composition of different blood fractions can be used to estimate erythrocyte EPA+DHA in a population with acute coronary syndrome.

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1. Introduction

The use of omega-3 blood biomarkers in clinical studies has increased since the introduction of the omega-3 index in 2004 as a possible risk factor for coronary heart disease [1]. The omega-3 index, which is the sum of the wt% of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of total fatty acids in erythrocytes has since been considered an emerging risk factor for cardiovascular disease and is being adopted as a biomarker of omega-3 fatty acid intakes for other disease and health outcomes particularly those related to pregnancy and psychiatry [2]. While the omega-3 index has greatly enabled the translation of research on omega-3 polyunsaturated fatty acids and health by establishing a standard system and blood targets, erythrocyte blood fatty acid analyses have not been universally adopted and other blood fractions such as plasma and whole are analysed despite the fact that target levels of omega-3 polyunsaturated fatty acids in these fractions are not established.

Erythrocyte content of EPA+DHA was selected as a potential new blood biomarker standard for determining omega-3 fatty acid status as it was believed that erythrocytes provided several distinct advantages over the EPA+DHA content of other blood fractions, specifically a longer half-life compared with plasma EPA+DHA, the existence of a lipid bilayer, and the absence of lipoproteins that increase fatty acid variation [1,3,4]. However, the collection and analysis of erythrocytes can introduce various practical issues involving collection, processing and storage [5] such that plasma and whole blood fatty acid determinations are completed instead. Some of the largest clinical studies examining blood fatty acids have used the plasma fraction [6–13] while whole blood use is increasing [14–18] due to gains in analytical throughput but also the comprehensive nature of the sample

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; GENESIS-PRAXY, (GENdEr and Sex determInantS of cardiovascular disease: from bench to beyond-Premature Acute Coronary SYndrome)

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[19,20]. The use of other fractions may also highlight challenges of the use of erythrocytes, as they are prone to oxidation during storage [21–23], and sample processing and analysis can be more complicated [20,24].

In the past, predictive equations to estimate plasma polyunsaturated fatty acid levels have been developed [25,26] but we are not aware of equations to translate EPA+DHA to different blood fractions or lipid pools within blood. The ability to convert fatty acid data from different blood fractions to erythrocyte EPA+DHA equivalents has the potential to be a useful tool for clinical and research applications. At an academic research level, it would enable researchers to better compare studies across various blood fractions. In the clinic, the assessment of omega-3 fatty acid status would be enabled as the analysis would not be dependent on a specific blood fraction that may or may not have been collected and/or stored properly. This is particularly relevant in management of acute clinical events such as a myocardial infarction. Therefore, we examined the fatty acid compositions of erythrocyte, plasma and whole blood samples at baseline from GENESIS-PRAXY (GENdEr and Sex determInantS of cardiovascular disease: from bench to beyond-Premature Acute Coronary SYndrome), a prospective, multicenter study examining acute coronary syndrome [27] and hypothesized that equations could be developed that would be able to predict levels of EPA+DHA in erythrocytes from plasma and whole blood fatty acids.

2. Patients and methods

2.1. Study population

Details on the study design and objectives of the GENESIS-PRAXY study [27] and sample collection [28] have been published previously. Briefly, it is a prospective study of patients aged 18–55 admitted to 24 centers (22 in Canada, 1 in the United States of America, and 1 in Switzerland) between 2009 and 2013. The McGill University Health Centre (Montreal, QC, Canada) received a multicenter ethical clearance and coordinated ethics approvals from each individual hospital ethics review board. In the present study, only participants with fatty acid composition determinations in plasma, erythrocytes and whole blood were included ($n=1104$). Participants were approached by a research nurse within 48 h of hospital admission and after consent, data was collected by self-administered questionnaires and medical chart reviews. Blood samples were collected and aliquots of plasma, erythrocytes and whole blood were prepared, frozen and stored locally at −80 °C until transported on dry ice to the McGill University Health Centre. The mean age of the study population was 48.0 ± 6.0 with 33% identifying themselves as a woman and 83% identifying themselves as Caucasian. Thirty-eight percent reported that they were currently smoking and 61% reported that they had completed some form of education beyond high school. Average total cholesterol (4.6 ± 1.2 mmol/L) and LDL cholesterol (2.8 ± 1.1 mmol/L) were desirable and near optimal, but mean HDL cholesterol (0.9 ± 0.3 mmol/L) was low and triacylglycerols (2.0 ± 1.2 mmol/L) were elevated relative to guidelines [29]. A subset of plasma samples ($n=40$) were also used for plasma lipid class analyses. These samples were purposively selected to cover the range of EPA+DHA levels determined in the initial fatty acid analyses.

2.2. Fatty acid analyses

The fatty acid composition of plasma, erythrocytes and whole blood were determined at the University of Waterloo using methods that have been published previously [24,28]. Samples were shipped to the University of Waterloo (Waterloo, ON, Canada) from the McGill University Health Centre on dry ice in four collated batches from 2009 to 2013 for timely analyses. Fatty acids were determined from the total lipids of plasma, erythrocytes and whole blood of all participants ($n=1104$). Lipids were extracted from plasma using the Folch, Lees and Sloane–Stanley method [30] and from erythrocytes using the modification by Reed et al. [31]. Both methods used chloroform/methanol with antioxidant (50 μg/mL solvent of 2,6-di-tert-butyl-4-methylphenol; Sigma-Aldrich, St. Louis, MO, USA) and internal standard (10 μg/sample of 22:3n-3 methyl ester; Nu-Chek Prep, Elysian, MN, USA) included during extraction. After vortexing, the samples were separated into organic and aqueous phases with the addition of 0.2 mol/L sodium phosphate buffer. The organic phase was collected, dried under nitrogen gas and dissolved in hexane for transesterification. Whole blood samples were directly transesterified with boron trifluoride and methanol with hexane (details below) and the internal standard (as above) added to the hexane.

For the subset study examining plasma lipid classes ($n=40$), plasma was extracted a second time with chloroform/methanol as described above but with 1,2-diheptadecanoyl-sn-glycero-3-phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL, USA), triheptadecanoin, heptadecanoate-cholesteryl ester and non-esterified heptadecanoic acid (Nu-Chek Prep) included as internal standards. The lipid extracts were then separated into lipid classes using thin layer chromatography [32]. Briefly, 20×20 cm^2 plates with a 6 nm silica gel layer (Whatman International Ltd., Maidstone, England) were used as the stationary phase and 60:40:2 heptane:diethyl ether:glacial acetic acid (v/v/v) was used as the mobile phase. After development, lipid class bands were visualized with 2,7-dicholroflourescein (Sigma-Aldrich, Oakville, Ontario), scraped and lipids were extracted from the silica gel with 2:1 chloroform/methanol (v/v). After extraction, the isolated lipid classes were dried under nitrogen and dissolved in hexane for transesterification.

The fatty acyls in the various lipid extracts in hexane were transesterified to fatty acid methyl esters with 14% boron trifluoride in methanol (Sigma-Aldrich, St. Louis, MO, USA) [33]. The fatty acid methyl esters were then analysed using fast gas chromatography [24] on a Varian 3900 gas chromatograph with a DB-FFAP capillary column (15 m × 0.10 mm diameter × 0.10 μm film thickness, J&W Scientific from Agilent Technologies, Mississauga, ON, Canada) with hydrogen as the carrier gas and nitrogen as the make-up gas. A temperature ramp protocol was used to optimize the resolution and peak shape of 32 fatty acid methyl esters that were routinely identified. Data was quantified using the internal standard response and data was expressed as concentrations (μg/200 mg erythrocytes, μg/100 μmol whole blood, plasma) and relative weight % of the total fatty acids.

2.3. Statistical analyses

All statistical analyses were completed using IBM® SPSS© Statistics v22 with statistical significance inferred when $p < 0.01$. The distribution of the fatty acid measures in blood were examined. Generally, the raw data tended to be leptokurtic and positively skewed as determined by the Kolmogorov–Smirnov and Shapiro–Wilk tests of normality and the skewness and kurtosis parameters as determined by functions in SPSS. However, visual inspection of residual plots in all the regression and correlation analyses performed indicated normal distributions, therefore data transformation was not used. The relationship between the sum of EPA and DHA in different blood fractions was examined using linear, quadratic and logarithmic curve estimation within SPSS. All linear relationships were then examined further using Pearson's correlation.

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