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Comparative effects of an acute dose of fish oil on omega-3 fatty acid levels in red blood cells versus plasma: Implications for clinical utility

William S. Harris, PhD*, Stephen A. Varvel, PhD, James V. Pottala, PhD, G. Russell Warnick, MS, MBA, Joseph P. McConnell, PhD

Health Diagnostic Laboratory, Inc, 737 N 5th Street, Suite 103, Richmond, VA 23219, USA (Drs. Harris, Varvel, Pottala, and McConnell; and Mr. Warnick); OmegaQuant Analytics, LLC, Sioux Falls, SD, USA (Dr. Harris); and Department of Internal Medicine, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD, USA (Drs. Harris and Pottala)

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BACKGROUND: Omega-3 fatty acid (n-3 FA) biostatus can be estimated with red blood cell (RBC) membranes or plasma. The matrix that exhibits the lower within-person variability and is less affected by an acute dose of n-3 FA is preferred in clinical practice.

OBJECTIVE: We compared the acute effects of a large dose of n-3 FA on RBC and plasma levels of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA).

METHODS: Healthy volunteers (n = 20) were given 4 capsules containing 3.6 g of n-3 FA with a standardized breakfast. Blood samples were drawn at 0, 2, 4, 6, 8, and 24 hours. The EPA + DHA content of RBC membranes and plasma (the latter expressed as a percentage of total FA and as a concentration) were determined. General linear mixed models were used to analyze the mean response profiles in FA changes over time for plasma and RBCs.

RESULTS: At 6 hours after load, the plasma concentration of EPA + DHA had increased by 47% (95% confidence interval [CI], 24% to 73%) and the plasma EPA + DHA percentage of total FA by 19% (95% CI, 4.7% to 36%). The RBC EPA + DHA percentage of composition was unchanged [–0.6% (95% CI, −2.6% to 1.5%)]. At 24 hours, the change in both of the plasma EPA + DHA markers was 10-fold greater than that in RBCs.

CONCLUSIONS: An acute dose of n-3 FA (eg, a meal of oily fish or fish oil supplements) taken within a day before a doctor's visit can elevate levels of EPA + DHA in plasma, whether expressed as a percentage or a concentration, but not in RBC membranes. Similar to hemoglobin A_{1c}, which is not affected by an acute glycemic deviation, RBCs provide a more reliable estimate of a patient's chronic EPA + DHA status than does plasma.

Potential conflicts of interest. All authors are employed by either Health Diagnostic Laboratory, Inc or OmegaQuant Analytics, LLC or both. Both of these companies offer red blood cell fatty acid testing, and the latter (primarily a research laboratory) also offers plasma assays. W.S.H. has received consulting fees and honoraria from GlaxoSmithKline and Amarin Corporation (companies with interests in pharmaceutical n-3 fatty acids). He is also on the scientific advisory boards of Omthera and Aker Biomarine Antarctic.

* Corresponding author.
E-mail address: Bharris@hdlabinc.com

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The Omega-3 Index is a validated biomarker of tissue membrane omega-3 (n-3) polyunsaturated fatty acid (PUFA) status.[1] The test analyzes the level of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) in red blood cell (RBC) membranes and expresses the result as a percentage of total fatty acids (FAs). The choice of RBCs was based on several factors, including previous literature that linked RBC EPA + DHA to risk of sudden cardiac death,[2] the ready availability of this biological membrane in blood samples, and the high correlation between RBC and myocardial EPA + DHA levels.[3,4] In addition, RBCs are a more stable, within-person biomarker of n-3 status than plasma, the latter having 4 times greater biological variability.[5] Just as the RBC-based hemoglobin A_{1c} assay is a more stable marker of glycemic status than fasting/fed serum glucose, the Omega-3 Index appears to be a more stable marker of n-3 status than plasma-based assays. Although stability has been documented over a 6-week period,[5] the *acute* effects of a dose of EPA + DHA, whether from supplements or fish, on n-3 biostatus has received only limited attention. A single dose of these FAs clearly raises plasma EPA and DHA levels, peaking at approximately 5 hours after administration,[6] but the effect of such a dose on RBC EPA and DHA content are unknown. The hypothesis tested in this study was that RBC EPA + DHA levels would be less affected by an acute load of n-3 FA than would plasma EPA + DHA levels.

There were both scientific and practical reasons for undertaking this study. Scientifically, it is not known how rapidly n-3 FA, on entering the plasma compartment in chylomicrons, become incorporated into cell membranes. Once in the nonesterified FA fraction, exchange is known to occur between DHA and RBC membranes, beginning approximately 8 hours after ingestion, with the predominant delivery form being albumin-bound DHA-lysophosphatidyl choline,[7] but the detailed time course in vivo is not clear. From a practical point of view, now that medical testing for n-3 FA status has become more common, clinicians have a choice of whether to use RBC- or plasma-based assays, and, for the latter, FA content can be expressed as a *percentage* of total FA or as a *concentration*. Different assays have different performance characteristics, fasting requirements, and sensitivities to perturbation with exogenous n-3 FA. Understanding these issues can help clinicians choose the appropriate testing method.

Methods

Subjects

Inclusion criteria were healthy male and female subjects of any race, older than 18 years, with body mass index (BMI; calculated as weight divided by height; kg/m^2) between 18 and 35. Subjects were recruited by a company-wide e-mail solicitation, and initially screened by telephone interview. Subjects who had taken fish oil supplements in the past 30 days, or who reported eating >1 entrée of "oily fish" (ie, salmon, herring, sardines, albacore tuna, mackerel) per week over the prior month were excluded. In addition, subjects with any known condition that would impair fat absorption (eg, cystic fibrosis, abetalipoproteinemia, chronic pancreatitis, pancreatic lipase deficiency syndrome) were also excluded. The first 20 subjects who qualified were e-mailed a copy of the informed consent document (which had been approved by the Copernicus Group Institutional Review Board). They subsequently attended an initial visit at which time the document was reviewed and informed consent was obtained by signature.

Procedures

The initial laboratory visit took place in the morning after at least an 8-hour fast at which time a baseline blood sample was drawn. Immediately after the blood draw, the subjects were given a standardized breakfast plus 4 enteric-coated fish oil capsules, each containing 900 mg of EPA (647 mg) + DHA (253 mg; Simply Natural Triple Strength; Sam's Club). This dose provided a total of 3.6 g of EPA + DHA as ethyl esters, which is equivalent to the n-3 FA dose one would get in approximately 6 ounces of farmed salmon. It is also approximately the dose for n-3 acid ethyl esters approved by the Food and Drug Administration; thus, there were no safety concerns. Subjects returned to the laboratory 2, 4, 6, 8, and 24 hours after the test meal for subsequent blood draws.

The standardized meals provided for breakfast, lunch, and dinner (and evening snack) contained no fish, and subjects were instructed to take no other fish oil pills during the study. The food was provided on the basis roughly of BMI, with subjects with a BMI < 27 receiving a total of 1800 kcal for the day and subjects with a higher BMI, 2200 kcal. Lunch and dinner were consumed after the blood draws at 4 and 8 hours, respectively. The final sample was drawn after a 10-hour overnight fast.

Laboratory methods

Blood was drawn into 5-mL ethylenediaminetetraacetic acid tubes and centrifuged immediately to separate RBCs from plasma. Aliquots of packed RBCs and plasma were transferred to cryovials and stored at −80°C until they were shipped on dry ice to OmegaQuant Analytics (Sioux Falls, SD) for analysis. Total cholesterol and triglycerides assays were performed with standard automated enzymatic methods on a Roche/Hitachi P-Modular system with Roche reagents (Roche Diagnostics, Indianapolis, IN). Low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were measured with direct enzymatic assays from Randox (County Antrim, United Kingdom) on a Roche/Hitachi P-modular system. Plasma glucose was measured with enzymatic methods on a