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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

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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.

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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

Beckman Coulter AU5842, hemoglobin A_{1c} was measured by ion-exchange high-performance liquid chromatography on a Bio-Rad Variant Turbo II, and complete blood cell count was measured on Beckman Coulter UniCel DxH800.

FA analysis

Samples were thawed, and an aliquot of plasma was combined (1:40 parts) with the derivatizing reagent [boron trifluoride in methanol (14%), toluene, and methanol (35:30:35 parts)], shaken, and heated at 100°C for 45 minutes. After cooling, 40 parts of both hexane and distilled water were added. After briefly vortexing, the samples were spun to separate layers, and an aliquot of the hexane layer that contained the FA methyl esters was extracted. Diheptadecanoyl-phosphatidyl choline was included in the plasma analysis as an internal standard for calculating concentrations. RBCs were analyzed similarly with the exception that only boron trifluoride in methanol (14%) was used for derivatization, and the samples were heated for only 10 minutes. Gas chromatography was performed with 100-m SP-2560 column in a Shimadzu 2010 instrument equipped with a flame ionization detector as described previously.[8] FAs were quantified by 3-point calibration curves and reported as percentage of composition (ie, each FA as a percentage of the total identified FAs) for both plasma and RBCs, and, in addition, as a concentration (μg/mL) in plasma. Coefficients of variation (CVs) for control samples were 2.5%, 2.0%, and 5.0% for these analytes, respectively.

Statistical methods

General linear mixed models were used with estimation to analyze the mean response profiles in FA changes over time for plasma and RBCs. The responses over time were all normalized to their baseline value, and because the distribution was right-skewed, a natural log transformation was used to improve normality and homoscedasticity of the residual errors. The repeated measures covariance structure that minimized Akaike Information Criterion[9] was selected as follows: Toeplitz for plasma concentration, first-order autoregressive for plasma percentage, and compound symmetry for RBC percentage. The Toeplitz and autoregressive structures are banded, with the later imposing a power lag function. The compound symmetry structure provides a single correlation between any 2 time points. The model parameter estimates were exponentiated and reported as percentage of changes from baseline. To determine which time points were different from baseline for each sample type, Dunnett adjustment was used to control for multiple comparisons. Dunnett adjusted P values $< .05$ were considered statistical significance. Analyses were performed with SAS software version 9.3. (SAS Institute).

Results

A total of 20 volunteers participated in this study. All laboratory values were within normal ranges (Table 1). The mean total energy consumed for the study day was 1936 ± 80.4 kcal, and the mean total fat intake was 66 ± 4.3 g (or 31% of kilocalories). A total of 19% of the day's kcal and fat (ie, 362 ± 77 kcal and 13 ± 4 g, respectively, excluding the kilocalories and fat from the supplements) were consumed at breakfast when the fish oil capsules were taken.

Baseline levels of the 5 major classes of FA (saturated, monounsaturated, n-6 and n-3 PUFAs, and *trans*) are presented in Table 2 along with the EPA + DHA values. The within-subject and between-subject variability (both expressed as the CV) differed substantially for each FA class by sample type. FA levels in RBCs had approximately 2-fold lower between-person variability than did plasma FA (percentage) for saturated, monounsaturated, and n-6 amounts; however, they were similar for n-3 PUFAs and *trans* FAs. Greater variability was observed in concentrations than in relative amounts. For example, the CV for saturated FAs expressed as concentrations was approximately 14-fold higher than the CV for this class in RBC membranes. For monounsaturated FAs, the CVs of these 2 expressions of FA status differed by 5-fold, and for the n-6 PUFA class, by 6-fold. For the n-3 PUFA the between-person variability across sample types was similar. Compared with the RBC-based metric, the within-person variability for the plasma percentage-based metric was 2- to 5-fold higher for all FA classes except *trans* FA, which was 10-fold higher. For the plasma concentration-based metric, it was 5- to 8-fold higher for all FA classes except

Table 1 Subject Characteristics (Men, n = 2; Women, n = 18)

	Mean	SD	Healthy Range
Age (y)	32	8.5	—
Race, n			
African American	9		—
White	9		—
Asian	2		—
Total cholesterol (mg/dL)	185	47.5	<200
LDL cholesterol (mg/dL)	113	46.9	<130
HDL cholesterol (mg/dL)	58	13.9	≥40
Triglycerides (mg/dL)	105	72.6	<150
Non-HDL cholesterol (mg/dL)	128	46.8	<130
Hemoglobin A_{1c} (%)	5.3	0.4	≤5.6
Glucose (mg/dL)	81	7.5	<100
White blood cells ($\times 10^3/\mu L$)*	6.8	2.7	4.0 to 10.5
Red blood cells ($\times 10^6/\mu L$)*	4.5	0.4	4.1 to 5.6
Hematocrit (%)*	41	4.3	36 to 50
Hemoglobin (g/dL)*	13	1.4	12.5 to 17.0
Platelets ($\times 103/\mu L$)*	251	46.2	140 to 415

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*n = 17.

Table 2 Abundance and Variability of Major FA Classes by Sample Type (n = 20)

	Saturated	Monounsaturated	Omega-6 PUFA	Omega-3 PUFA	*trans* FA	EPA + DHA
Red blood cell						
Mean (% of total FA)	40.4	13.4	36.9	7.5	1.4	4.8
SD (% of total FA)	0.9	1.2	1.5	1.6	0.3	1.5
Within subject CV (%)*	1.5	1.4	2.0	2.7	1.8	3.1
Between subject CV (%)	2.3	9.0	4.0	21.4	21.2	31.8
Plasma						
Mean (% of total FA)	28.4	20.4	45.8	3.5	1.1	2.3
SD (% of total FA)	1.7	3.3	4.1	0.9	0.3	0.8
Within subject CV (%)*	3.3	5.9	3.4	11.6	18.1	15.7
Between subject CV (%)	5.9	16.3	9.1	25.0	29.9	34.7
Plasma						
Mean (μg/mL)	834.2	613.6	1325.5	98.9	33.3	64.1
SD (μg/mL)	260.4	277.9	334.5	27.1	13.0	20.8
Within subject CV (%)*	12.1	14.9	9.2	16.7	21.9	19.3
Between subject CV (%)	31.2	45.3	25.2	27.4	39.1	32.4

CV, coefficient of variation; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; PUFA, polyunsaturated fatty acid; SD, standard deviation.

*Within-subject CV was calculated over the 6 time points; all other measures are at baseline.

the *trans* and monounsaturated FAs, which were >10-fold higher.

For the FA metric of primary interest in this study, EPA + DHA, the individual subject data (and group means with 95% confidence interval [CI]) for all 3 sample types across the 24-hour period are shown in Figure 1. When expressed as percentage of changes from baseline, both of the plasma-based metrics showed significant increases over the study period, but the RBC-based metric did not (Fig. 2). At 6 hours after load, the plasma concentration of EPA + DHA had increased by 47% (95% CI, 24% to 73%), the plasma EPA + DHA as a percentage of total FA by 19% (95% CI, 4.7% to 36%), and the RBC EPA + DHA percentage composition by −0.6% (95% CI, −2.6% to 1.5%). At 24 hours all sample types had significant increases in EPA + DHA of 28%, 30%, and 2.8%, respectively.

Discussion

The purpose of this study was to compare the response of plasma-based with RBC-based markers of n-3 FA status to a pharmacologic dose of n-3 FAs. We found that the 24-hour excursion for plasma (expressed as both percentage and concentration) was significantly greater than that for RBCs. In plasma the peak n-3 FA concentration was observed at 6 hours, whereas the peak as a percentage of total FA was seen at 24 hours. RBC EPA + DHA levels were increased from baseline only at 24 hours, but by an order of magnitude less than plasma (approximately 3% vs 30% elevated).

In a previous study we examined the within-person biological variability in EPA + DHA by sample type over 6 weeks in volunteers with a stable n-3 FA intake.[5] EPA + DHA levels as a percentage of total FA were 10 times less variable when measured in RBCs (CV_{within} = 1.3%) than in plasma (CV_{within} = 13.6%) (Fig. 3). These values, measured over weeks, were similar to those observed here (2.7% and 15.7%, respectively) which were measured over hours after a large n-3 FA load. Despite the differences in within-person variability, the mean levels for both (fasting) plasma and RBCs were stable over that 6-week study. Therefore, in research studies done in large populations, either metric can provide an estimate of n-3 FA status, but in the clinical setting, where visit-to-visit comparisons are most important, the RBC-based metric is preferred. Another problem with plasma-based metrics is their sensitivity to fasting status. In the earlier study,[5] the effects of a high-fat meal (with no fish oil) on EPA + DHA levels in RBCs and plasma (again, both expressed as a percentage of total FA) were assessed. Four hours after the meal, no change was observed in the RBC metric, but an 11% decrease (P = .05) was seen in the plasma metric. The latter was caused by the increase in FAs from the meal in the plasma which reduced the n-3 fraction as a percentage of the total. In the present study, the percentage of EPA + DHA in plasma increased by approximately 20% after the meal. This is because a large amount of n-3 FA was given with the test meal. Although a large dose of EPA + DHA as given here can begin to affect the RBC level after 24 hours, it generally takes 12 to 16 weeks for the EPA + DHA level in RBCs to reach a new steady state after a sustained increase in intake[10]; plasma (phospholipid) levels stabilize in approximate 6 weeks.[11] The findings of the present study compliment these longer term data and indicate that the RBC-based metric is not only more stable over time when n-3 FA intake is unchanging but is also essentially unaffected by an acute dose of n-3 FA.

The difference in the FA composition of plasma and RBCs is well known.[12–14] Relative to the former, the latter

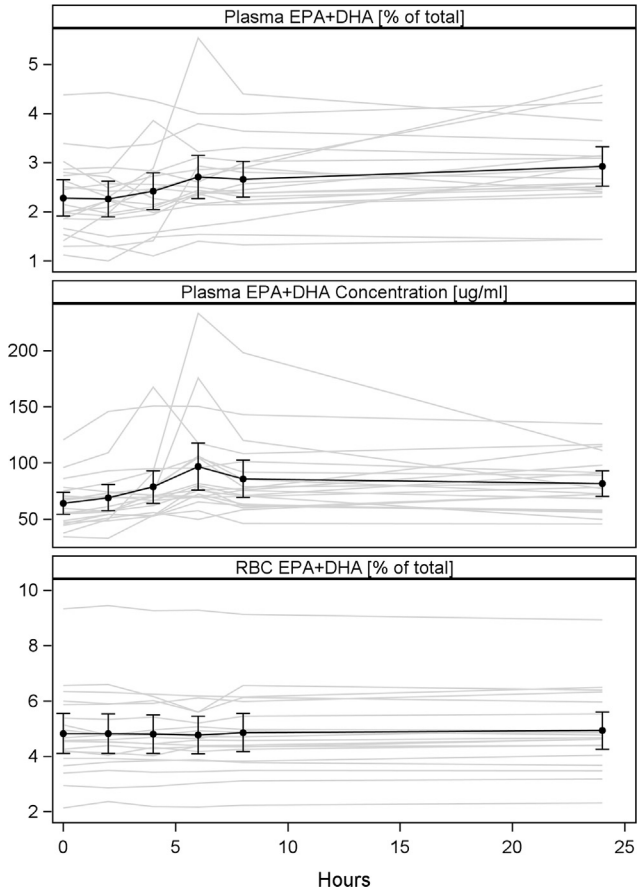

Figure 1 Effects of a single dose of EPA + DHA (3.4 g) taken with breakfast on absolute EPA + DHA levels in plasma (expressed as a percentage of total fatty acids, top; and expressed as concentrations, middle) and in RBCs (expressed as a percentage of total fatty acids; bottom) over 24 hours. Individual patient responses (n = 20) are shown (gray lines) and group means with 95% confidence intervals (black lines). DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RBC, red blood cell.

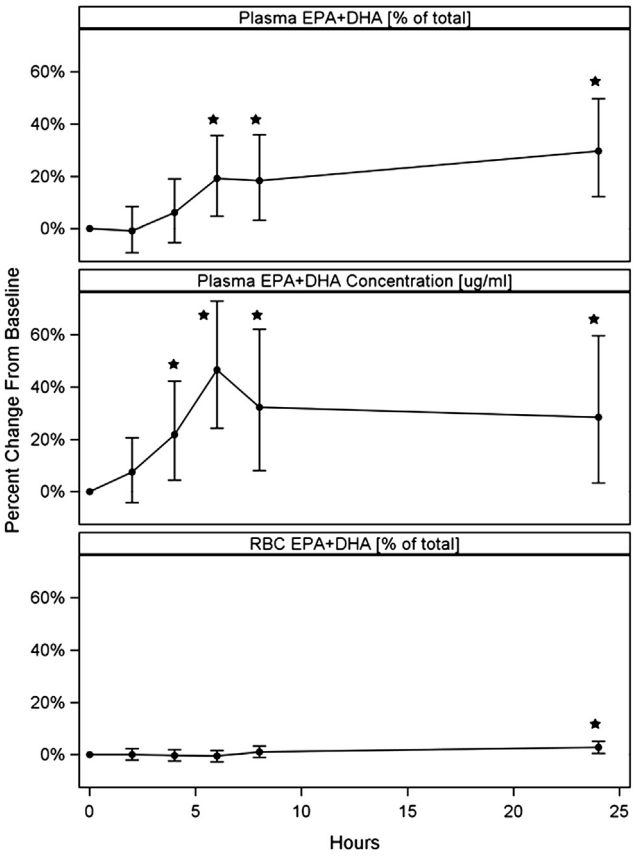

Figure 2 Effects of a single dose of EPA + DHA (3.4 g) taken with breakfast on the percentage of change from baseline (hour 0) in EPA + DHA levels in plasma (expressed as a percentage of total fatty acids, top; and expressed as concentrations, middle) and in RBCs (expressed as a percentage of total fatty acids; bottom) over 24 hours (n = 20). Mean estimate and 95% Dunnett-adjusted confidence intervals are shown. *Difference from baseline, $P < .05$. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RBC, red blood cell.

are enriched in PUFAs (both n-6 and n-3) because as membranes, they are comprised almost entirely of phospholipids, the lipid class that carries the greatest proportion of long-chain PUFAs.[12] With respect to their responses to changes in n-3 FA intakes, all blood-based pools (and cardiac tissue[3,4]) become enriched with higher intakes to roughly equal extents.[15]

Although recent meta-analyses of randomized trials have questioned the value of n-3 FA supplementation for reducing the risk of coronary heart disease,[16,17] the epidemiologic evidence for a benefit from higher intakes of n-3 FA (which are better correlated with RBCs than plasma[18]) are robust.[19,20] Indeed, several studies have specifically linked RBC n-3 FA levels with cardiovascular disease (CVD) outcomes. Most notably, in 1995 Siscovick et al[2] were the first to document a strong correlation with RBC EPA + DHA and primary cardiac arrest. In 2002, Albert et al[21] showed that whole blood EPA + DHA (which is highly correlated with RBC levels[1]) was an independent predictor of risk of sudden cardiac death. Building on this foundation, the Omega-3 Index was proposed in 2004 as a marker of risk of death from CVD.[1,22] This particular assay method has now been widely used in a variety of studies that examined not only CVD but also depression and cognitive function.[23–39] Others that used different analytical methods have reported RBC n-3 FA associations with other conditions. For example, in a large cohort in China, RBC DHA was strongly and inversely associated with risk of multiple forms of chronic disease.[40] RBC FA patterns have been related to atrial fibrillation,[41] longevity,[42] literacy/attention deficit hyperactivity disorder,[43] breast cancer in Japan,[44] prostate-specific antigen levels in Jamaica,[45] cognitive function,[46,47] hostility,[48] eating disorders,[49] and dyslexia.[50] Relatively few studies have examined the relations between plasma FA percentage of composition and CVD outcomes, and most studies have come from Japan.[51–58] Only one study has compared the percentage of composition of n-3 FA in plasma and RBCs as predictors of coronary heart disease, in this case for nonfatal myocardial infarction.[58] The former was generally better than the

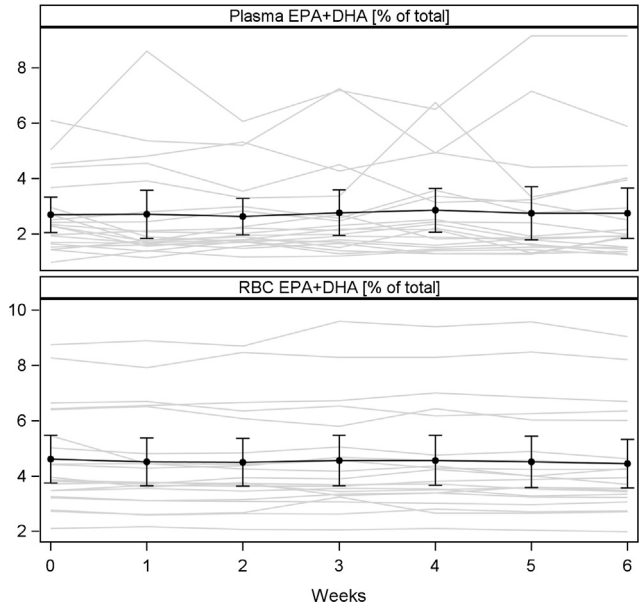

Figure 3 Levels of EPA + DHA in whole plasma (upper) and RBCs (lower; both in percentage of composition) measured in the fasting state once a week for 6 weeks in 21 healthy volunteers. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RBC, red blood cell. Data from Harris and Thomas.[5]

latter, but their RBC assay produced EPA and DHA levels that were roughly equal, which is highly suspect as DHA is typically 6-8 fold higher than EPA in RBCs (24-26). This suggests co-elution of a contaminant with EPA in the analysis, making the RBC data questionable in this study. A recent study from the Cardiovascular Health Study that focused on plasma phospholipid n-3 FA percentage (another analyte that correlates well with the RBC metric[15]) reported a significant inverse relationship between n-3 FA levels and total mortality.[59]

As alluded to earlier, many of the RBC-based studies[23–39] have used the identical laboratory method,[8] whereas a variety of methods were used in the plasma-based studies. Without formal interlaboratory comparison, there is no guarantee that values with one method will agree with values of another method; even RBC-based methods vary in output.[60] It has been shown that methodologic differences in just the pre-chromatography phase of FA processing can lead to different values.[61]

The results of this study have practical implications. For example, most patients are told to increase their intake of oily fish or to start taking fish oil supplements after a blood test reveals suboptimal n-3 FA levels. Although some will comply, others will not but may choose to take a single large dose of fish oil within 24 hours before their appointment in an effort to improve their blood levels and thereby appear to have been compliant. When their blood is drawn at the appointment, their plasma EPA + DHA level will indeed be elevated and could easily mislead the physician, but the RBC level should be unchanged, reflecting the true chronic condition.

Finally, there is a place for both plasma and RBC n-3 FA analyses (and for whole blood, if it is the only sample type available[21,28]). As noted earlier, both could potentially be used in population research studies (if fasting status is known) because mean levels are reasonably stable, and these 2 markers are well correlated (particularly as percentages, $r = 0.90$; not as well for RBC percentage vs plasma concentration, $r = 0.73$; unpublished observations). Plasma also responds more quickly to a change in n-3 FA intake than does the RBC pool.[11] The RBC method, however, has at least 4 characteristics that favor its use in the clinical setting: low within-subject variability, resistance to artificial elevation after an acute n-3 FA dose, much longer half-life,[10] and insensitivity to fasting status. In addition, the amount of published research that links RBC n-3 FA levels with clinically relevant outcomes is greater than that for plasma. Hence, RBC-based therapeutic targets have a clinical relevance not enjoyed by plasma-based targets.

Conclusion

In conclusion, the RBC fraction appears to be the preferred sample type for the assessment of n-3 FA biostatus.

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References

1. Harris WS, von Schacky C. The omega-3 index: a new risk factor for death from coronary heart disease? *Prev Med*. 2004;39:212–220.
2. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *J Am Med Assoc*. 1995;274:1363–1367.
3. Harris WS, Sands SA, Windsor SL, et al. Omega-3 fatty acids in cardiac biopsies from heart transplant patients: correlation with erythrocytes and response to supplementation. *Circulation*. 2004;110:1645–1649.
4. Metcalf RG, Cleland LG, Gibson RA, et al. Relation between blood and atrial fatty acids in patients undergoing cardiac bypass surgery. *Am J Clin Nutr*. 2010;91:528–534.
5. Harris WS, Thomas RM. Biological variability of blood omega-3 biomarkers. *Clin Biochem*. 2010;43:338–340.
6. Davidson MH, Johnson J, Rooney MW, Kyle ML, Kling DF. A novel omega-3 free fatty acid formulation has dramatically improved bioavailability during a low-fat diet compared with omega-3-acid ethyl esters: the ECLIPSE (Epanova compared to Lovaza in a pharmacokinetic single-dose evaluation) study. *J Clin Lipidol*. 2012;6:573–584.
7. Brossard N, Croset M, Normand S, et al. Human plasma albumin transports [13c]docosahexaenoic acid in two lipid forms to blood cells. *J Lipid Res*. 1997;38:1571–1582.
8. Harris WS, Pottala JV, Vasan RS, Larson MG, Robins SJ. Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in older Americans. *J Nutr*. 2012;142:1297–1303.

9. Akaike H. Information theory as an extension of the maximum likelihood principal. In: Petrov B, Csaki F, editors. Second International Symposium of Information Theory and Control. Budapest: Akademia Kiado, 1973. p. 267–281.
10. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res*. 1997;38:2012–2022.
11. Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids. *Am J Clin Nutr*. 2007;86:1621–1625.
12. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res*. 2008;47:348–380.
13. Yanagisawa N, Shimada K, Miyazaki T, et al. Polyunsaturated fatty acid levels of serum and red blood cells in apparently healthy Japanese subjects living in an urban area. *J Atheroscler Thromb*. 2010;17:285–294.
14. Rise P, Eligini S, Ghezzi S, Colli S, Galli C. Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans. *Prostaglandins Leukot Essent Fatty Acids*. 2007;76:363–369.
15. Browning LM, Walker CG, Mander AP, et al. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. *Am J Clin Nutr*. 2012;96:748–758.
16. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. *JAMA*. 2012;308:1024–1033.
17. Kwak SM, Myung SK, Lee YJ, Seo HG. Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials. *Arch Intern Med*. 2012;172:686–694.
18. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in us women. *Am J Clin Nutr*. 2007;86:74–81.
19. Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: an updated meta-analysis of seventeen cohort studies. *Public Health Nutr*. 2012;15:725–737.
20. Xun P, Qin B, Song Y, et al. Fish consumption and risk of stroke and its subtypes: accumulative evidence from a meta-analysis of prospective cohort studies. *Eur J Clin Nutr*. 2012;66:1199–1207.
21. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med*. 2002;346:1113–1118.
22. Harris WS. The omega-3 index: from biomarker to risk marker to risk factor. *Curr Atheroscler Rep*. 2009;11:411–417.
23. Salisbury AC, Harris WS, Amin AP, Reid KJ, O'Keefe JH Jr., Spertus JA. Relation between red blood cell omega-3 fatty acid index and bleeding during acute myocardial infarction. *Am J Cardiol*. 2012;109:13–18.
24. Harris WS, Kennedy KF, O'Keefe JH Jr, Spertus JA. Red blood cell fatty acid levels improve GRACE score prediction of 2-yr mortality in patients with myocardial infarction. *Int J Cardiol*. 2013;168:53–59.
25. Shearer GC, Pottala JV, Spertus JA, Harris WS. Red blood cell fatty acid patterns and acute coronary syndrome. *PLoS ONE*. 2009;4:e5444.
26. Harris WS, Pottala JV, Lacey SM, Vasan RS, Larson MG, Robins SJ. Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study. *Atherosclerosis*. 2012;225:425–431.
27. Ladesich JB, Pottala JV, Romaker A, Harris WS. Membrane level of omega-3 docosahexaenoic acid is associated with severity of obstructive sleep apnea. *J Clin Sleep Med*. 2011;7:391–396.
28. Pottala JV, Garg S, Cohen BE, Whooley MA, Harris WS. Blood eicosapentaenoic and docosahexaenoic acids predict all-cause mortality in patients with stable coronary heart disease: the Heart and Soul study. *Circ Cardiovasc Qual Outcomes*. 2010;3:406–412.
29. Block RC, Harris WS, Reid KJ, Spertus JA. Omega-6 and trans fatty acids in blood cell membranes: a risk factor for acute coronary syndromes? *Am Heart J*. 2008;156:1117–1123.
30. Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. *Atherosclerosis*. 2007;197:821–828.
31. Kim YJ, Jeong DW, Lee JG, et al. Omega-3 index and smoking in patients with acute ST-elevation myocardial infarction taking statins: a case-control study in Korea. *Lipids Health Dis*. 2012;11:43.
32. Park Y, Lim J, Lee J, Kim SG. Erythrocyte fatty acid profiles can predict acute non-fatal myocardial infarction. *Br J Nutr*. 2009;102:1355–1361.
33. Park Y, Park S, Yi H, et al. Low level of n-3 polyunsaturated fatty acids in erythrocytes is a risk factor for both acute ischemic and hemorrhagic stroke in Koreans. *Nutr Res*. 2009;29:825–830.
34. Park Y, Oh SH, Rhee MY. Association between 24-hour ambulatory blood pressure and erythrocyte n-3 polyunsaturated fatty acids in Korean subjects with hypertension. *Nutr Res*. 2010;30:807–814.
35. Baghai TC, Varallo-Bedarida G, Born C, et al. Major depressive disorder is associated with cardiovascular risk factors and low omega-3 index. *J Clin Psychiatry*. 2010;72:1242–1247.
36. Moyers B, Farzaneh-Far R, Harris WS, Garg S, Na B, Whooley MA. Relation of whole blood n-3 fatty acid levels to exercise parameters in patients with stable coronary artery disease (from the Heart and Soul study). *Am J Cardiol*. 2011;107:1149–1154.
37. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, Whooley MA. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. *JAMA*. 2010;303:250–257.
38. Ali S, Garg SK, Cohen BE, Bhave P, Harris WS, Whooley MA. Association between omega-3 fatty acids and depressive symptoms among patients with established coronary artery disease: data from the Heart and Soul study. *Psychother Psychosom*. 2009;78:125–127.
39. Farzaneh-Far R, Harris WS, Garg S, Na B, Whooley MA. Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: the Heart and Soul study. *Atherosclerosis*. 2009;205:538–543.
40. Wang Y, Crawford MA, Chen J, et al. Fish consumption, blood docosahexaenoic acid and chronic diseases in Chinese rural populations. *Comp Biochem Physiol A Mol Integr Physiol*. 2003;136:127–140.
41. Viviani Anselmi C, Ferreri C, Novelli V, et al. Fatty acid percentage in erythrocyte membranes of atrial flutter/fibrillation patients and controls. *J Interv Card Electrophysiol*. 2010;27:95–99.
42. Puca AA, Andrew P, Novelli V, et al. Fatty acid profile of erythrocyte membranes as possible biomarker of longevity. *Rejuvenation Res*. 2008;11:63–72.
43. Milte CM, Parletta N, Buckley JD, Coates AM, Young RM, Howe PR. Eicosapentaenoic and docosahexaenoic acids, cognition, and behavior in children with attention-deficit/hyperactivity disorder: a randomized controlled trial. *Nutrition*. 2012;28:670–677.
44. Kuriki K, Hirose K, Wakai K, et al. Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids in Japanese. *Int J Cancer*. 2007;121:377–385.
45. Ritch CR, Brendler CB, Wan RL, Pickett KE, Sokoloff MH. Relationship of erythrocyte membrane polyunsaturated fatty acids and prostate-specific antigen levels in Jamaican men. *BJU Int*. 2004;93:1211–1215.
46. Chiu CC, Frangou S, Chang CJ, et al. Associations between n-3 PUFA concentrations and cognitive function after recovery from late-life depression. *Am J Clin Nutr*. 2012;95:420–427.
47. Heude B, Ducimetiere P, Berr C. Cognitive decline and fatty acid composition of erythrocyte membranes-the EVA Study. *Am J Clin Nutr*. 2003;77:803–808.

48. Watari M, Hamazaki K, Hirata T, Hamazaki T, Okubo Y. Hostility of drug-free patients with schizophrenia and n-3 polyunsaturated fatty acid levels in red blood cells. *Psychiatry Res*. 2010;177:22–26.
49. Swenne I, Rosling A, Tengblad S, Vessby B. Omega-3 polyunsaturated essential fatty acids are associated with depression in adolescents with eating disorders and weight loss. *Acta Paediatr*. 2011;100: 1610–1615.
50. Cyhlarova E, Bell JG, Dick JR, Mackinlay EE, Stein JF, Richardson AJ. Membrane fatty acids, reading and spelling in dyslexic and non-dyslexic adults. *Eur Neuropsychopharmacol*. 2007;17:116–121.
51. Nakamura T, Azuma A, Kuribayashi T, Sugihara H, Okuda S, Nakagawa M. Serum fatty acid levels, dietary style and coronary heart disease in three neighbouring areas in Japan: the Kumihama study. *Br J Nutr*. 2003;89:267–272.
52. Nogi A, Yang J, Li L, et al. Plasma n-3 polyunsaturated fatty acid and cardiovascular disease risk factors in Japanese, Korean and Mongolian workers. *J Occup Health*. 2007;49:205–216.
53. Oda E, Hatada K, Katoh K, Kodama M, Nakamura Y, Aizawa Y. A case-control pilot study on n-3 polyunsaturated fatty acid as a negative risk factor for myocardial infarction. *Int Heart J*. 2005;46: 583–591.
54. Sekikawa A, Curb JD, Ueshima H, et al, ERA JUMP (Electron-Beam Tomography, Risk Factor Assessment Among Japanese and U.S. Men in the Post-World War II Birth Cohort) Study Group. Marine-derived n-3 fatty acids and atherosclerosis in Japanese, Japanese-American, and white men: a cross-sectional study. *J Am Coll Cardiol*. 2008;52: 417–424.
55. Siguel EN, Lerman RH. Altered fatty acid metabolism in patients with angiographically documented coronary artery disease. *Metabolism*. 1994;43:982–993.
56. Ueeda M, Doumei T, Takaya Y, et al. Serum n-3 polyunsaturated fatty acid levels correlate with the extent of coronary plaques and calcifications in patients with acute myocardial infarction. *Circ J*. 2008;72: 1836–1843.
57. Ferrucci L, Cherubini A, Bandinelli S, et al. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab*. 2006;91:439–446.
58. Sun Q, Ma J, Campos H, et al. Blood concentrations of individual long-chain n-3 fatty acids and risk of nonfatal myocardial infarction. *Am J Clin Nutr*. 2008;88:216–223.
59. Mozaffarian D, Lemaitre RN, King IB, et al. Plasma phospholipid long-chain omega-3 fatty acids and total and cause-specific mortality in older adults: a cohort study. *Ann Intern Med*. 2013;158: 515–525.
60. Harris WS, von Schacky C, Park Y. Standardizing methods for assessing omega-3 fatty acid biostatus. In: McNamara RK, editor. The Omega-3 Fatty Acid Deficiency Syndrome: Opportunities for Disease Prevention. Hauppauge, NY: Nova Science Publishers, Inc: 2013. p. 385–398.
61. Bocking C, Nockher WA, Schreiner M, Renz H, Pfefferle PI. Development and validation of a combined method for the biomonitoring of omega-3/-6 fatty acids and conjugated linoleic acids in different matrices from human and nutritional sources. *Clin Chem Lab Med*. 2010;48:1757–1763.