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Erythrocyte omega-3 fatty acids increase and linoleic acid decreases with age: Observations from 160,000 patients

William S. Harris*, James V. Pottala, Stephen A. Varvel, James J. Borowski, Jennie N. Ward, Joseph P. McConnell

Health Diagnostic Laboratory, Inc., Richmond, VA, United States

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ABSTRACT

Background: The fatty acid (FA) composition of the red blood cell (RBC) has been reported to provide prognostic information regarding risk for coronary heart disease (CHD). In particular, the Omega-3 Index (RBC eicosapentaenoic acid + docosahexaenoic acid, EPA + DHA) has been shown to be independently and inversely related to risk for sudden cardiac death and for acute coronary syndromes. Higher linoleic acid (n-6) and lower *trans* FA levels have also been associated with improved CHD outcomes. Accordingly, the RBC FA panel has recently been introduced in routine clinical laboratory testing.
Objective: The purpose of this study was to define age- and gender-based norms for RBC FA levels.
Methods: RBC FA profiles from about 160,000 patients (48% from males, 52% from females) were measured at Health Diagnostic Laboratory. These data were used to create age decade and gender-specific norms (percentiles). FA values were expressed as a percent of total identified FA.
Results: Compared to men, women generally had higher C18 *trans* levels, and between the ages of 10–29 years, they had DHA and lower EPA levels. Among the major FA classes, saturated (41% of total) and *trans* (~0.85%) fats did not vary appreciably by age, whereas monounsaturated fats tended to rise slightly. Of the two major n-6 polyunsaturates, arachidonic and linoleic acids, the former was unchanged across decades (16.4% abundance) whereas the latter decreased by about 2 percentage points (13.0–11.1%). The overall median Omega-3 Index was 4.5%, and across the decades it increased by about 1.5 percentage points. The Omega-3 Index and linoleic acid stabilized after age 70.
Conclusion: Whereas RBC saturated, mono- and polyunsaturated FA levels are generally stable across the lifespan, there is a shift in the composition of the latter, with an increase in the Omega-3 Index and a decrease in linoleic acid. Higher DHA and lower EPA levels in younger women is consistent with enhanced conversion of EPA to DHA during the early reproductive years. The availability of RBC FA norms will facilitate research into the relationships between altered FA status and human disease, and will help physicians evaluate the n-3 FA status of their patients.

1. Introduction

Red blood cell (RBC) membrane fatty acid composition is determined by a combination of diet and metabolism. All major classes of fatty acids are found in RBC—saturated, monounsaturated, *trans* unsaturated, and polyunsaturated (both n-3 and n-6 families). Much interest has focused on the marine n-3 fatty acids, eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively; the sum of which is the Omega-3 Index) [1], as reduced levels in both the diet and in RBC membranes have been associated with a higher cardiovascular disease (CVD) risk profile [2], increased risk for CVD [3,4] and neuropsychiatric [5] diseases, accelerated cellular aging [6] and early mortality [7]. Since these associations are largely independent of other known risk factors, and because the Omega-3 Index is an easily, safely and inexpensively treated risk factor, clinical laboratories have begun to offer testing. Accordingly, it is important to establish age- and sex-based norms against which practitioners can evaluate their patients. The purpose of this study was to define those values for a large and unselected cohort of patients whose blood was submitted for testing at a commercial laboratory.

2. Methods

2.1. Subjects

Data for this study was obtained from blood samples submitted for testing to Health Diagnostic Laboratory, Inc. (HDL,

* Corresponding author. Tel.: +1 877 443 5227 × 2226; fax: +1 804 343 2704.
E-mail address: bharris@hdlabinc.com (W.S. Harris).

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Richmond, VA). RBC fatty acid data were extracted without any linked patient identifiers. Age and gender were the only demographic data available. IRB approval for these types of studies (using de-identified and aggregated laboratory data) was obtained from the Copernicus Group (Durham, NC).

In an effort to address the question of how representative of the general population this convenience patient sample was, RBC fatty acid composition of an age- and gender-matched subset of HDL data was compared to that of the Framingham cohort [2]. Additional comparisons were made with recent data from the National Health and Nutrition Examination Survey (NHANES) derived from 1806 randomly selected US adults between 2003 and 2006 [8]. As the NHANES reported *plasma* fatty acid *concentrations* and not *RBC* fatty acid *percent composition*, the former were converted into plasma fatty acid percent composition (a closer surrogate of the RBC metric) by summing the plasma concentrations across all fatty acids and then calculating each fatty acids' percent of total. Although plasma and RBC fatty acid compositions are numerically different, for the essential n-6 and n-3 fatty acids they are relatively well-correlated ($r=0.6$–0.8) [9]. Trends in plasma essential fatty acid composition across the lifespan from NHANES were then compared to trends observed in the HDL cohort.

2.2. Laboratory methods

RBC fatty acid composition was analyzed according to the HS-Omega-3 Index® methodology as modified from Harris et al. [10] Fatty acid methyl esters were generated from erythrocytes by transesterification with boron trifluoride and analyzed by gas chromatography. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of RBCs. Omega-3 index results are given as EPA plus DHA expressed as a percentage of total identified fatty acids after response factor correction (based on calibration curves) was applied to each fatty acid. The CV for the Omega-3 Index assay was $<3.5\%$.

2.3. Statistical methods

Percentiles were calculated for individual and groups of fatty acids. Percentiles were also calculated by age decade, and gender for the Omega-3 Index. Stratified (by age decade and gender) random sampling was used to match a subset of the HDL patients to the Framingham cohort for comparison of fatty acid values.

Since most of the fatty acid distributions were right-skewed, a natural logarithm transformation was used to improve the normality and homogeneity assumptions required for parametric general linear models. The mean of the log-transformed data (i.e., geometric mean) was tested for gender differences ($\geq 5\%$ relative effect size) in each age decade using *t*-tests. Linear trends ($\geq 1\%$ relative change per decade) were tested using piece-wise linear regression with a break (i.e., knot) at 70 years. The estimated effects were exponentiated and reported as relative percent changes in fatty acids. Due to the very large sample size, meaningful effect sizes (noted above) were used in combination with a Bonferroni adjusted critical level of 0.05/10 tests=0.005 for each fatty acid to ascribe statistical significance. Analyses were performed using SAS® software (version 9.2; SAS Institute).

3. Results

Information on RBC fatty acid composition was available from about 160,000 individual patients measured between July 2011 and April 2012 (see Table 1 for sample sizes by decade and gender). Teenagers through nonagenarians were represented, with females slightly outnumbering males. Population ranges and variability in RBC fatty acid content were estimated using percentile values (from the 1st to the 99th) for the major fatty acids of interest the population coefficients of variation (Table 2). Total saturated and total polyunsaturated fatty acids had the least between-person variability, with coefficients of variation $<5\%$.

Table 1
Sample size by gender and decade of life.

Decade	Total	Men	Women
10s	1153	503	650
20s	5948	2580	3368
30s	14,464	7017	7447
40s	28,545	14,393	14,152
50s	39,430	19,368	20,062
60s	37,991	18,196	19,795
70s	22,695	10,442	12,253
80s	8561	3581	4980
90s	984	351	633
Total	159,771	76,431 (48%)	83,340 (52%)

Mean levels of arachidonic acid (the primary n-6 fatty acid in RBC membranes), total polyunsaturated and saturated fatty acid compositions remained very stable across the lifespan, and there were no gender differences (Fig. 1, total saturated not shown). Monounsaturated fatty acid levels increased a small extent over the first seven decades. The mean levels of the 18-carbon (C18) *trans* fatty acids were relatively flat through the 60s but increased slightly thereafter. Total n-6 polyunsaturated fatty acids decreased between the teens and 70s due primarily to a 3% per decade fall in linoleic acid levels. Total n-3 fatty acids increased across the same time fame because of increases in EPA (13% per decade), docosapentaenoic acid (DPA) (3% per decade), and DHA (6% per decade). There was a significant decrease in the mean levels of EPA (-9% per decade) and DPA (-1% per decade) after age 70. The net effect on the Omega-3 Index was an overall increase of about 7% per decade up thru 70 years, with little change thereafter. The percentile distributions for the Omega-3 Index by decade of life for men and women combined are shown in Fig. 2.

Gender-based differences were observed in all the n-3 and in the C18 *trans* fatty acids (Fig. 1). Mean EPA and DPA levels were reduced in women compared to men through the 40s and DHA was increased for women in their teens and twenties. ALA levels were higher in women than men by about 5% relative amounts across all ages. During the 30s through 50s, *trans* levels were slightly higher in women vs. men.

The Framingham cohort was used as a relatively normative comparator group and included 3196 individuals, 9% of which were minorities and 45% of which were male. Their mean (SD) age was 66 (9) years. The Framingham erythrocyte samples were obtained during 2005–2007, whereas the HDL samples were collected between 2011 and 2012. Framingham cohort and the HDL subgroup that was matched by age and gender with it, had very similar erythrocyte fatty acid profiles (Table 3). The largest differences were seen in a few very low-abundance fatty acids including C18:1 *trans* oleic acid and the sphingolipid-associated fatty acids [C24:0 (lignoceric acid) and C24:1 (nervonic acid)].

4. Discussion

The primary question addressed in this study was, "do RBC fatty acid patterns vary with age and/or gender?" As such, this was a descriptive study that included data from about 160,000 people with ages between 10 and 99 years. This is the largest