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 (C240.85%) 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.

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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,
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 concentrations 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 (≥ 5% relative effect size) in each age decade using t-tests. Linear trends (≥ 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%.

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 frame 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 (−5% 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
dataset describing RBC fatty acid patterns yet reported, and as such, can have value in establishing norms for use in the clinic, at least in the US. These individuals were all being evaluated by their physicians for cardiovascular risk factors as a part of normal clinical care, and therefore, these findings may apply only to such individuals, and the extent to which they describe the “typical American” is unknown. A comparison with the Framingham cohort was undertaken to shed some light on this question, and the remarkable similarity in RBC fatty acid composition between these two cohorts – despite being collected 6 years apart – suggests that the data from the HDL patient cohort is reasonably generalizable in the US.

### 4.1. Relations with age

The mean levels of the major fatty acid classes (saturated, monounsaturated and polyunsaturated) were generally constant across the lifespan. This consistency and the low between-person

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### Table 2

Percentiles of major RBC fatty acids (N=159,771).

<table>
<thead>
<tr>
<th>Fatty Acids (as a proportion of total)</th>
<th>1st</th>
<th>5th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>95th</th>
<th>99th</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-linolenic acid (n-3)*</td>
<td>0.10</td>
<td>0.10</td>
<td>0.12</td>
<td>0.14</td>
<td>0.16</td>
<td>0.23</td>
<td>0.30</td>
<td>30.6</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (EPA)</td>
<td>0.19</td>
<td>0.25</td>
<td>0.36</td>
<td>0.50</td>
<td>0.84</td>
<td>1.94</td>
<td>3.19</td>
<td>84.4</td>
</tr>
<tr>
<td>Docosapentaenoic acid (n-3)</td>
<td>1.61</td>
<td>1.88</td>
<td>2.25</td>
<td>2.53</td>
<td>2.88</td>
<td>3.61</td>
<td>4.17</td>
<td>20.3</td>
</tr>
<tr>
<td>Docosahexaenoic acid (n-3)</td>
<td>1.87</td>
<td>2.33</td>
<td>3.17</td>
<td>4.04</td>
<td>5.18</td>
<td>7.02</td>
<td>8.50</td>
<td>34.0</td>
</tr>
<tr>
<td>Omega-3 Index</td>
<td>2.20</td>
<td>2.70</td>
<td>3.60</td>
<td>4.50</td>
<td>6.00</td>
<td>8.80</td>
<td>11.10</td>
<td>38.8</td>
</tr>
<tr>
<td>Linoleic acid (n-6)</td>
<td>1.95</td>
<td>2.40</td>
<td>3.15</td>
<td>3.95</td>
<td>4.95</td>
<td>6.95</td>
<td>8.95</td>
<td>30.3</td>
</tr>
<tr>
<td>Saturated fats</td>
<td>38.50</td>
<td>39.40</td>
<td>40.50</td>
<td>41.30</td>
<td>42.40</td>
<td>43.70</td>
<td>44.50</td>
<td>3.2</td>
</tr>
<tr>
<td>Monounsaturated fats</td>
<td>12.90</td>
<td>13.70</td>
<td>14.90</td>
<td>15.70</td>
<td>16.60</td>
<td>18.00</td>
<td>19.30</td>
<td>8.3</td>
</tr>
<tr>
<td>C18 Trans</td>
<td>0.46</td>
<td>0.57</td>
<td>0.74</td>
<td>0.87</td>
<td>1.03</td>
<td>1.36</td>
<td>1.73</td>
<td>28.3</td>
</tr>
</tbody>
</table>

CV=between patients coefficient of variation.

* N = 126,628.
variability suggest homeostatic mechanisms are at work. Although consumed in relatively large quantities in the diet, the saturated and monounsaturated fatty acids are also synthesized de novo which may explain why both plasma and RBC membrane content of these fatty acids do not correlate strongly with intake [11].

As regards the polyunsaturated fatty acids, the overall constant mean level obscures changes that occur within the two subgroups: the n-6 and n-3 fatty acids. The major n-6 fatty acid in the RBC membrane, arachidonic acid (~16% of total), was remarkably stable over time and had little inter-individual variability (CV = 11%). Arachidonic is derived from the diet and synthesis from linoleic acid (although synthesis rates have been reported to be < 1% [12]).
Marked changes in dietary linoleate, either up or down, do not affect plasma phospholipid arachidonic acid levels [13]. The NHANES-reported linoleate acid intakes of around 16 g per day represents about 7% of total energy intake, and given that required intakes of this essential fatty acid are 1–2% of energy [14], dietary linoleate availability is not rate limiting for arachidonate synthesis, therefore metabolic factors must determine membrane AA levels. For its part, linoleate acid levels were inversely associated with age, decreasing about 3% per year up until age 70. A similar trend was seen in the plasma fatty acid data from NHANES [8]. The plasma linoleic acid percent dropped across the lifespan, e.g., from 32.7% (20–39 years) to 31.6% (40–59 years) to 30.5% (>59 years). The reasons for the inverse relations between age and RBC linoleic acid levels, also observed by others [15–17], are not clear. One obvious explanation would be a change in linoleate intake. However, dietary data from NHANES (2009–2010) show stable intakes of this fatty acid between the teens and the 50s; intakes start to decrease at about the same time that RBC linoleate levels stabilize (cf. Figs. 3 and 1) [18]. Other potential explanations for this effect are discussed below.

In concert with the decreasing linoleic acid content in RBCs we observed increasing EPA and DHA levels. This relationship between age and long-chain n-3 fatty acid levels (i.e., the Omega-3 Index) has been reported several times before with smaller data sets [19–26]. Plasma EPA+DHA levels from NHANES show a similar tendency to rise across the lifespan, e.g., from 1.4% (20–39 years) to 1.5% (40–59 years) to 1.6% (>59 years). A variety of forces could be operating here. Most obviously, increasing intakes (both dietary and supplementary) of EPA and DHA could contribute. The amount of EPA and DHA consumed by the individuals in the current study is unknown; however the most recent NHANES dietary data [18] (2009–2010) indicate that the intake of EPA and DHA from food (not supplements, which is not available) does appear to increase with age through the 50s, similar to the trend seen in the RBC (cf. Figs. 3 and 1). Consistent with this observation, some investigators have reported that the rise in this biomarker with age is linked with dietary changes [15,27], whereas others have reported the rise to be independent of n-3 intake [2,15–17,19]. The rise in the Omega-3 Index with age could be secondary to an increasing ALA intake, but this is also generally stable across the lifespan (Fig. 3) as were RBC ALA levels (Fig. 1). While very large increases in dietary linoleic acid (e.g., increasing from 5 g/d to 36 g/d) can reduce the incorporation of supplemental EPA and DHA into plasma and tissues [28,29], a more physiologic increase (e.g., going from 8 g/d to 16 g/d) does not affect membrane n-3 content [30]. Hence, the variations in population linoleic acid intake across the lifespan would not be expected to impact n-3 fatty acid levels. On the other hand, in supplementation studies with EPA and DHA, linoleic blood levels are often reduced as the n-3 fatty acid levels increase [31–34], so higher n-3 fatty acid levels can lower linoleic levels. This is likely due to competition between these polyunsaturated fatty acids for incorporation into the 2-position of phospholipids. On balance, it seems more likely that the rise in the n-3 fatty acids is the cause of the decrease in linoleate, and not vice versa.

Cunnane and colleagues have explored the n-3 fatty acid—age relationship in some detail. They have reported that elderly individuals respond to supplemental EPA and DHA with a greater rise in plasma DHA (RBC not tested) than young people [35], and that labeled DHA is cleared from plasma lipids (especially from the triglyceride and non-esterified fatty acid compartments) more slowly in the elderly, but overall oxidation of DHA is not different between old and young [36]. Consequently, changes in underlying fatty acid metabolism may be playing a role. Another possible explanation for a rising Omega-3 Index with age is “the attrition of the susceptible.” Since a lower blood n-3 fatty acid level is associated with increased risk for death in randomized trials [37–39], and in some [40,43] but not all [40,41,44–47] prospective cohort studies, it is possible that the rise in the Omega-3 Index with age is an artifact, a result of the premature demise of younger individuals with lower levels. Hence, aging may not raise blood n-3 levels; but higher n-3 levels may promote longevity [6,7]. This explanation is suspect, however, since the apparent rise with age is most evident (and steady) between the teens and the 60s, and is flat from the 70s on. Since most deaths occur in the latter group, one would expect that a mortality effect would be most striking in the older, not younger, people. At present, the reason for the direct association between age and the Omega-3 Index is an increasing intake with age.

Trans fatty acid levels (about 1.0% of total RBC fatty acids) are relatively unrelated to age, and are considerably lower than they were in Framingham both in 1999 (2.4%) and in 2006 (1.8%) [10]. This suggests that the continuing efforts of the food industry (under government and health organization pressure) to reformulate vegetable oils to remove trans fatty acids has been successful in reducing the burden of these fatty acids in tissues.

Greater intrinsic (i.e., metabolic) control of membrane fatty acid composition would be reflected in lower inter-individual variability in RBC fatty acid levels (which can be expressed as coefficients of variation, CV). Greater dietary influence on composition would be reflected in higher CVs. This fits the observations made here where the largest CVs were seen with the n-3 and trans fatty acids, and the smallest with saturated, monounsaturated and omega-6 fatty acids (especially arachidonic acid).

4.2. Relations by gender

The higher population CVs observed for the n-3 and trans fatty acids, are partially due to gender differences. There were differences for EPA and DHA: in young women (teens and 20s), EPA was lower and DHA was higher than in young men. Women have been reported to have higher DHA levels in some studies [2,24,27,48] but not in others [15,26,49]. Conversion of alpha-linolenic acid to DHA may be higher in young women than men [50], likely driven by sex hormones [51]. In older women, the current study did not detect a gender difference in the Omega-3 Index whereas there was a small difference in Framingham, where the mean age is 67 years [2], which suggests that hormonal effects may not be completely responsible. Of course there could be a dietary etiology as well, and NHANES data do show lower EPA and DHA intakes in young women vs. men (Fig. 3). However, differences in DHA intake in this age range do not explain the higher RBC DHA content in young women. However, NHANES intake data are not adjusted for body weight.

This study has strengths and limitations. Among the former are large sample size encompassing nine decades of life with an even mix of men and women. Limitations include the lack of information on dietary and other clinical/demographic data from these subjects. The RBC fatty acid values observed here are likely specific to the US, and possibly other westernized countries; they would not be normative for other countries, particularly in Japan [21] and Korea [52], where fish consumption is higher.

5. Conclusion

RBC fatty acid data observed from over 160,000 outpatients in late 2011–early 2012 provide an estimate of “normal” values for these analytes across the lifespan for a US population. Although most fatty acid levels are stable, the Omega-3 Index is directly, and linoleate acid is inversely, related to age. Trans fatty acid levels in this study are considerably lower than they were in earlier
studies suggesting that continuing national efforts to reduce consumption of trans fats and to remove trans fatty acids from food products has met with some success. Young women have higher DHA levels than young men, which likely represents enhanced conversion of EPA to DHA during the early reproductive years. The availability of RBC fatty acid normative values will facilitate research into the relationships between altered fatty acid status and human disease, and will help physicians evaluate the n-3 fatty acid status of their patients across age and gender groups.

References


