Prostaglandins, Leukotrienes and Essential Fatty Acids xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

The Omega-6:Omega-3 ratio: A critical appraisal and possible successor

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ARTICLE INFO

Keywords:
Omega-3 fatty acids
Omega-6 fatty acids
Eicosapentaenoic acid
Docosahexaenoic acid
Linoleic acid
Arachidonic acid
Biomarkers
Omega-3 index

ABSTRACT

The well-known health effects of the long-chain, marine omega-3 (n-3) fatty acids (FAs) has led to a growing interest in the prognostic value that blood levels of these FAs might have vis-à-vis cardiovascular and neurocognitive diseases. The measurement and expression of n-3 FA levels is not straight-forward, however, and a wide variety of means of expression of n-3 FA status have been used in research and clinical medicine. This has led to considerable confusion as to what "optimal" n-3 FA status is. The n-6:n-3 ratio has enjoyed relatively widespread use, but this apparently simple metric has both theoretical and practical difficulties that have contributed to misunderstandings in this field. Just as the once-popular polyunsaturated:saturated FA ratio has largely disappeared from the nutritional and medical literature, it may be time to replace the n-6:n-3 ratio with a newer metric that focuses on the primary deficiency in Western diets – the lack of eicosapentaenoic and docosahexaenoic acids (EPA and DHA). The Omega-3 Index (red blood cell EPA + DHA) has much to recommend it in this regard.

1. Introduction

There is a growing interest in exploring the relationships between fatty acid (FA) status and clinically important health outcomes [1]. These include cardiac disease [2–4], stroke [5], diabetes [6], cognitive function [7], and aging [8–10]. However, analysis of FAs is much more complicated than it is for other biomarkers like cholesterol or glucose. The latter analytes circulate in plasma as single molecular species whose concentrations can be easily measured by long-ago standardized enzymatic methods, and optimal levels are clearly defined after decades of research, either as a risk factor for a disease [e.g., coronary heart disease (CHD), the former] or as a diagnostic for disease (e.g., diabetes, the latter). There are several reasons why FA testing is more challenging. First, there are many different FA species, typically organized into groups based on the *number of double bonds* they contain [0, saturated; 1, monounsaturated; >1, polyunsaturated (PUFA); or >2, highly unsaturated (HUFA)]. FAs within the latter 2 groups are further segregated based on the *position of the terminal double bond* into the omega (n)-6 and n-3 groups. But even these are not homogeneous groupings as the physiological functions of FAs within each class may differ depending on *carbon chain length* and *orientation* of the double bonds (*cis* vs. *trans*). Beyond the differences in molecular species, FA status can be measured in *multiple lipid pools* – from whole blood to blood cells (red, white or platelet) to whole plasma or plasma lipid classes or even subclasses. In general, the same FAs are found in all lipid pools but always in unique relative proportions peculiar to that pool [11]. Like cholesterol and glucose, FA levels can be expressed in molar or mass units. Finally, regardless of the pool analyzed, FA status can be expressed as *composition* (each as a percent of total) or as *concentration* (mass/volume or cell count). Partly because of these challenges, defining a "high risk" FA level that can be used clinically has been difficult.

In the 1960s the "P:S ratio" became popular as the ratio of dietary/plasma polyunsaturated vs. saturated FAs was inversely related to serum cholesterol levels [12]. This metric became obsolete as Mensink et al. demonstrated the illogic of its underlying assumptions [13]. These included physiologic differences between two FAs from the same class (e.g., one saturated FA – palmitic – raised cholesterol but another – stearic – did not), and physiological similarities between two FAs from different classes (e.g., *trans* monounsaturated FAs proved to have even more detrimental effects on serum lipids than saturated FAs). What's more, *cis* monounsaturated FAs had clear beneficial effects on a classic CHD risk marker – the ratio of total to high density lipoprotein cholesterol – and they were not included in the P:S ratio. The confluence of these advances in science led to the eventual demise of the P:S ratio.

The discovery in the late 1970s of the potential health benefits of the marine n-3 FAs (eicosapentaenoic and docosahexaenoic acids, EPA and DHA) by Bang and Dyerberg in Greenland Inuits [14] sparked an avalanche of studies on these novel FAs. The realization that EPA and arachidonic acid (AA, n-6) competed as substrates for several enzymes critical to hemostasis, vascular reactivity, and inflammation suggested that some kind of ratio of the n-6 to n-3 FAs in both the diet and the

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https://doi.org/10.1016/j.plefa.2018.03.003
Received 7 March 2018; Accepted 9 March 2018

blood might be a convenient way to conceptualize the overall "omega" status. This comported well with the pioneering studies of Holman and colleagues showing that the shorter chain n-6 and n-3 FAs (linoleic and alpha-linolenic acids, LA and ALA) also competed with each other for desaturase and elongase enzymes in the production of AA and EPA/DHA. These findings further supported a "competition" mindset and provided a conceptual underpinning for the n-6:n-3 ratio. The discovery that aspirin blocked the cyclo-oxygenase mediated conversion of AA to a variety of pro-inflammatory eicosanoids fed into the idea that AA was itself pro-inflammatory, and soon, ALL n-6 FAs were painted with that brush. So the n-6 PUFAs became "bad" and the n-3 PUFAs "good," and a ratio seemed a reasonable way to simply represent the body's potential inflammatory response to an insult [15]. However, in recent years, the complex biochemistry of the eicosanoids (and docosanoids and octadecanoids) has become clearer, with some n-6 FA metabolites being pro- and others anti-inflammatory [16], so the class itself can no longer be so simply regarded as pro-inflammatory. In addition, beneficial (not detrimental) effects of the primary dietary n-6 FA, LA, have been repeatedly observed [17–19]. These new findings began to erode the view that PUFA biology could be summed up in one simple ratio. Now, based on a further appreciation of both its conceptual problems and flawed assumptions [20], calls to abandon the use of this ratio have become more frequent [20–23]. These concerns are discussed below, and an alternative approach to expressing PUFA status is suggested.

2. The n-6:n-3 ratio is imprecise and non-specific

> *"What is the usefulness of the ratio of n-6 to n-3, which is good divided by good?"* [23]

The components of the n-6:n-3 ratio are rarely defined. The ratio is formed by summing all of the n-6 FAs in either the diet or a biological sample and dividing it by the sum of all the n-3 FAs. As simple as this sounds, even here we find ambiguity since "all" depends on how many FAs are actually quantified in a given study. The major n-6 FA in the diet and plasma is LA followed by AA. There are trace amounts of gamma-linolenic (GLA) in the diet, but its blood levels are very low. Then there are a variety of other n-6 metabolites not in the diet, but present in the blood, again at low levels. These would include dihomo-gamma linolenic acid (DGLA), adrenic acid (ADA), eicosadienoic acid (EDA) and docosapentaenoic acid (DPAn-6). The major n-3 FA in the western diet is ALA, but the n-3 FA in greatest abundance in blood and most tissues is DHA, followed by DPAn-3 and EPA. Depending on the analytical conditions, greater or fewer individual FA species may be measured. Using RBC data from a random set of 50 individuals measured in our laboratory, if one includes the seven n-6 FAs listed above and the four n-3 FAs, one gets a ratio of 7.8 in RBCs. If instead only the "important" or "major" n-6 FAs (LA and AA) and n-3 FAs (ALA, EPA and DHA) are included, the ratio is 9.3. The lack of a standardized definition of which FAs constitute both the numerator and the denominator of the ratio is an obvious weakness.

The lipid pool in which the ratio is calculated is not defined. Again using data from our laboratory as an example, when the ratio is measured in RBCs and in plasma cholesteryl esters from the same 50 people, the ratio ranges from 3.5% in RBCs to nearly 29% in plasma (Table 1). The ratio in RBCs also differs considerably from that in platelets (2.7 vs. 6.3) [24], and to get even more granular, each different class of phospholipids present in cell membranes has its own characteristic FA composition, and thus n-6:n-3 ratio. RBC phosphatidyl-choline, -ethanolamine, -serine and -inositol have ratios of 12, 2, 2, and 4.5, respectively [25]. A further challenge on this point has to do with measurements made in whole plasma/serum since this matrix contains an undefined mixture of 4 lipid classes (phospholipids, triglycerides, cholesteryl esters, and free FAs) each with its own FA signature [11], and except for the free FAs, these are all carried in unique proportions in 3 different lipoprotein particles (very low-, low- and high-density lipoproteins).

Table 1
Differences in the n-6:n-3 ratio by lipid pool measured in the same 50 random blood samples in the author's laboratory.

Lipid compartment	n-6:n-3 ratio (wt%)
Whole blood	7.8
Red blood cell	3.5
Whole plasma/serum	9.1
Non-esterified FAs	4.0
Cholesteryl esters	19.7
Phospholipids	4.9
Triglycerides	6.5

The n-6:n-3 ratios in patients with various dyslipidemias can thus be affected by variations in serum levels of each lipid class [11].

The means of expression of FA abundance is not defined. FA status can be expressed in molar or mass terms (whether as concentration or percent compositions). For example, the RBC ratio is 11.2 based on mol % expression and 10.2 on weight%.

Identical ratios can be calculated from an endless variety of individual FA levels. This weakness, raised earlier [20], can be illustrated by considering a RBC membrane with 30% LA + AA and 8.3% EPA + DHA. (The latter value is called the Omega-3 Index, and a value of 8.3% is within the optimal cardioprotective zone [26,27]). The n-6:n-3 ratio of this sample would be 3.6. Virtually the same ratio would be calculated for a sample containing 18% LA + AA and 5% EPA + DHA (which is near the undesirable zone of < 4% for the Omega-3 Index). Hence, both high and low risk status could have the same ratio. (To be fair, this is only a theoretical concern since in human biology, the RBC membrane PUFA content is held constant, so when the n-3 FA level increases, the n-6 level decreases; they cannot both decrease to any appreciable extent [28]).

Coherent dietary advice cannot be given based only on the n-6:n3 ratio. Based on NHANES data, the average n-6:n-3 ratio of the American diet is about 10 [29]. Some have advocated the consumption of a diet with a ratio of 1 – the presumed ratio of the ancient human diet [30]. Putting aside for a moment the problem of the implicit presumption of metabolic equivalence of each FA within each class (discussed below), there are at least five ways to lower a ratio that is "too high" (Table 2), and the physiological consequences of each approach differs. For example, lowering *both* n-3 and n-6, the latter more than the former (approach 5 in the Table) is clearly less healthy than simply raising the n-3 intake (approach 2). Ratio-thinking distracts from the almost universal need for individuals with a "high" ratio to simply raise their EPA + DHA intake, not lower their n-6 intake. Thus, it can be challenging for a clinician who is "ratio-focused" to make rational and healthy dietary recommendations.

3. Use of the n-6:n-3 ratio is based on invalid assumptions

In addition to problems of imprecision and non-specificity, there are at least four assumptions underlying the use of the n-6:n-3 ratio that are, if not completely false, at least highly debatable. This thin evidentiary foundation contributes to the disutility of the metric.

Assumption 1: Omega-6 FAs have adverse effects on cardiovascular health. More precisely, LA – because it can be converted to AA which can then be metabolized to pro-inflammatory eicosanoids – increases the chronic inflammatory status of the body which predisposes to

Table 2
Five ways to lower the n-6:n-3 ratio [19].

Approach	1	2	3	4	5
n-3 FAs	↑	↑	↑↑	→	↓
n-6 FAs	↓	→	↑	↓	↓↓
Ratio	↓	↓	↓	↓	↓

chronic diseases. Although this assumption is potentially supported by the re-analysis of two vegetable oil feeding trial in the 1970s–80s for CVD [31,32] and by a prospective cohort study linking higher LA intake to depression in women [33], there is a large body of literature on human disease outcomes that shows either neutral or beneficial effects of dietary intakes or blood levels of LA on risk for CVD [17,18,34] and type 2 diabetes mellitus [35,36]. Hence, LA is not bad, it's good for cardiometabolic health.

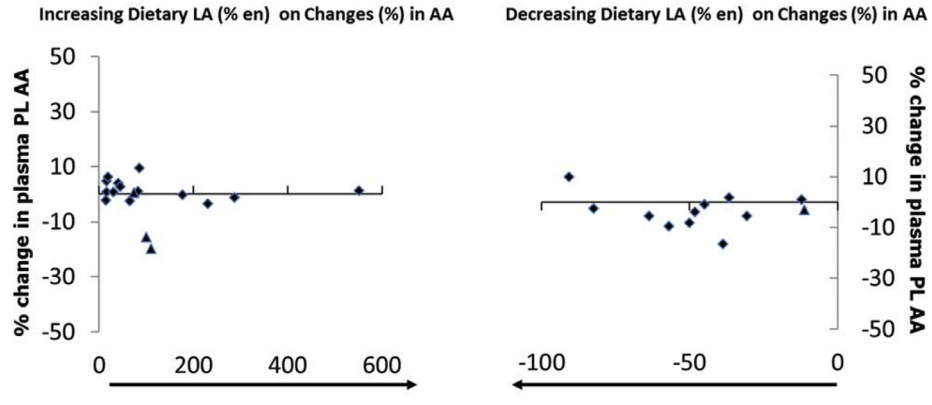
Assumption 2: Omega-6 FAs are proinflammatory. As alluded to above, the increased risk for disease supposedly associated with a high n-6:n-3 ratio is classically attributed to the numerator being pro- and the denominator being anti-inflammatory [15,37]. However, this view, which might have been reasonable in the 1970s, is now far too simplistic [16], and enjoys little to no direct support from studies in humans [38–40]. Higher LA levels have actually been associated with reduced inflammatory status [41–43]. Even supplementation with AA (which raises serum AA levels, an effect not shared by LA supplementation; see below) does not stimulate an inflammatory response [44]. Higher inflammatory status can be seen in settings where EPA and DHA levels are low [45] (i.e., the ratio is high), but the problem is not the presence of the n-6 FAs but the absence of the n-3s.

Assumption 3: Lowering the intake of LA will lower tissue AA levels. At the core of concerns about high n-6:n-3 ratios is that AA is proinflammatory, and that the way to reduce the inflammatory stress on the body is to cut back on the consumption of AA's precursor, LA. (Typical daily intakes in the US are around 15 g of LA and 0.15 g of AA [29]). A review of studies that either raised LA intakes or lowered them and then followed the effects on serum phospholipid AA levels found that the latter are not affected by changes in the former [46] (Fig. 1). Indeed, tracer studies have shown that $<0.2\%$ of dietary LA is converted to AA [47]. If lowering AA serum levels was a desirable goal (which is questionable based on meta-analysis which found that lower AA levels was associated with *increased* risk for CHD [2]), then the most effective way to do it would be to increase the intake of EPA and DHA [28].

Assumption 4: All n-6 FAs are equally "bad" and all n-3 FAs are equally "good." Because of the lack of specificity in the ratio's pooling of all FAs from each PUFA class into a single value, the implicit assumption is that each of the seven n-6 FAs commonly measured has equal biological function, and the same is true of the n-3 FAs, i.e., ALA has the same physiological effects as EPA, DPA and DHA. This is clearly untrue if for no other reason than the myriad of oxylipins synthesized from multiple PUFAs have widely varying effects [16,48]. This point has also recently been forcefully demonstrated for the n-6 FAs as well. Delgado et al. correlated the risk for death from CVD and any cause over 10 years with RBC n-6 FA levels in a large German cohort [49]. As seen in Fig. 2, overall, the n-6 FAs are not significantly related with risk for CVD in the multivariable adjusted model. However, when specific n-6 FAs are examined individually for their relationships with these outcomes, an interesting pattern emerges: three are inversely associated with risk for death (LA, GLA and DGLA), one is neutral (AA), and two others are directly associated (ADA and DPA). This strongly suggests that this family of FAs cannot be distilled down into one metric where all FAs in the set hold the same relations with risk (for CVD death, in this case). The meta-analysis by Chowdhury et al. on circulating FA levels and risk for incident CHD makes a similar point. LA (highest vs. lowest tertile) was not associated with risk, but AA was favorably related. As regards the n-3 FAs, in Chowdhury there was no association with CHD events for circulating ALA levels, but risk was reduced by 25% for EPA + DHA [2]. Hence for both classes of PUFAs, the assumption that all members have the same "risk value" is false.

4. A possible successor to the n-6:n3 ratio?

If the n-6:n-3 ratio is now outmoded, what could take its place as a biomarker of "omega" status? In 2004, I and my colleague Clemens von Schacky proposed a metric called the "Omega-3 Index" which is the RBC EPA + DHA content expressed as a weight percent of total RBC membrane FAs. This metric is not burdened with several of the concerns raised above: it is specific as to matrix (RBC), and to the FA components (EPA + DHA), and to expression form (wt %). Also, the actions needed to correct an undesirable (low) level are unambiguous: consume more EPA + DHA. As described above, too much LA and AA is not the problem in Western cultures with high rates of chronic disease – the problem is the consumption of too little EPA and DHA, and thus a metric that directly focuses on these FAs properly diagnoses the deficiency, and the action needed to rectify it is clear. The Omega-3 Index is highly responsive to changes in EPA + DHA intake [28,50], and the levels in RBCs reflect those of other tissues [51,52]. One potential weakness of the Omega-3 Index, however, is its summing of EPA and DHA. This is because some studies have shown differential effects of these two marine n-3 FAs on risk factors or different associations with disease [53–58]. On the other hand, many have also shown that the Index itself does provide independent predictive information for a variety of diseases and so fulfills its primary purpose as a nutritional status screening test. Because the n-3 component of the n-6:n-3 ratio is the primary driver of the ratio (e.g., [10,59]), the latter is highly correlated with the Omega-3 Index (Fig. 3). It is also strongly correlated with other proposed metrics such as the n-3 HUFA:total HUFA ratio proposed by Lands [60], and (to a lesser extent) the AA/EPA commonly reported in Japanese studies [61–63] (Fig. 3). Accordingly, the correlations of these ratio-based metrics with disease outcomes reported in some studies (e.g., [61,64,65] are likely explained by the n-3 component alone. For example, in the Women's Health Initiative Memory Study, RBC EPA + DHA was significantly and inversely associated with risk for



Diamonds = NS findings; Triangles = Significant findings. Adapted from [46].

Fig. 1. Non-effects of either raising or lowering dietary linoleic acid (LA) intakes on serum phospholipid arachidonic acid (AA) content.

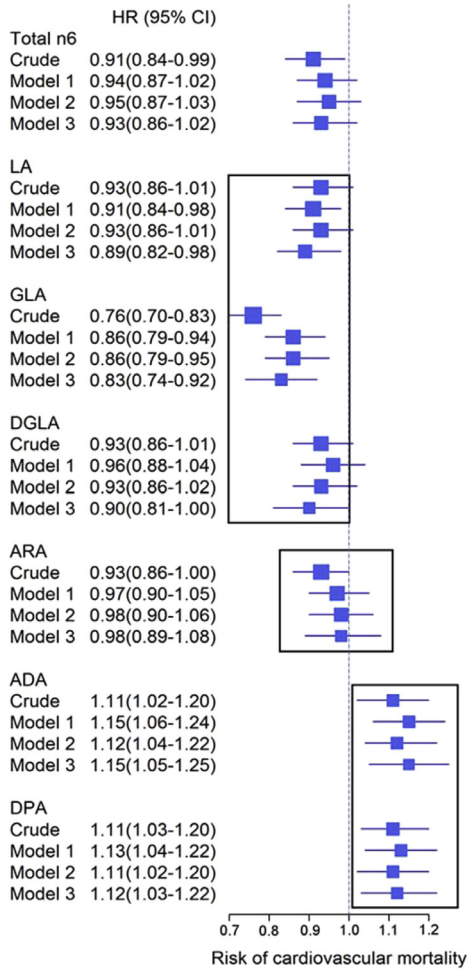


Fig. 2. Hazard ratios per 1 SD FA increase by 3 stastical models for RBC n-6 FAs and cardiovascular disease mortality in the LURIC study [49]. Reprinted with permission.

death from any cause (Table 3) whereas LA and AA levels were not. The n-6:n-3 ratio was, however, and given the similar (but inverse) hazard ratios, it is clear that the denominator completely explained the ratio's association with risk [10]. The failure of many researchers to provide data on both the numerator and denominator when they report the n-6:n-3 ratio adds to the confusion in this field.

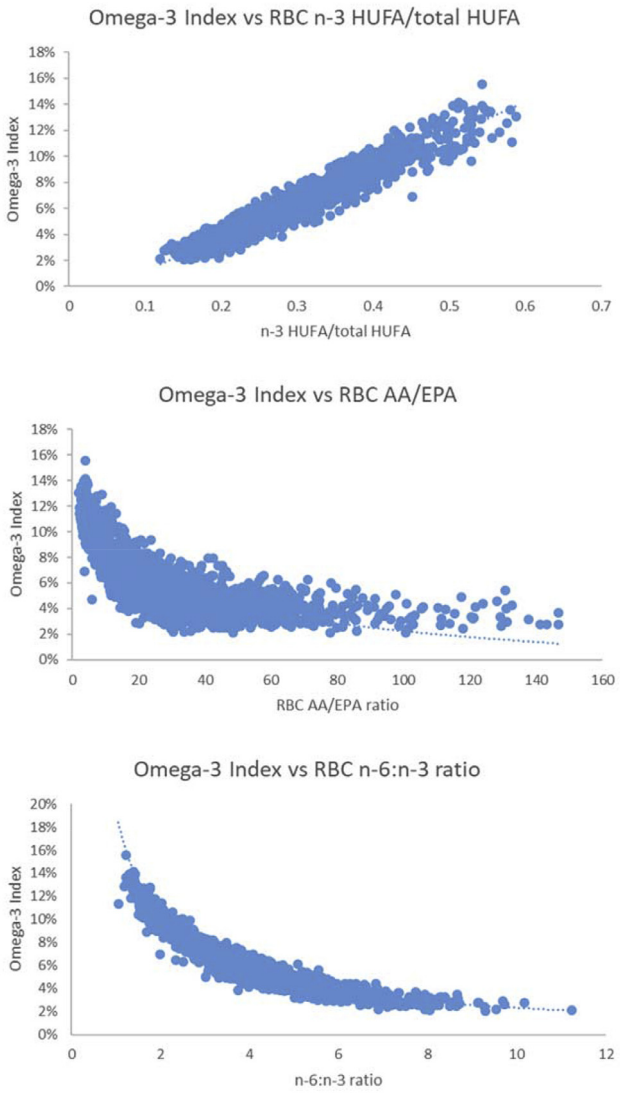
The Omega-3 Index was chosen by Stark et al. [66] to express worldwide n-3 status and by Health Canada for that country's national health survey [67]. The single largest dataset published on circulating FA status in humans, which included about 160,000 individuals in the USA, utilized the Omega-3 Index [68]. The Omega-3 Index has been used in multiple observational cohort and interventional studies around the world. It has been associated with lower risk for coronary disease [2] sudden cardiac death [69,70], acute coronary syndromes [71], all-cause mortality [9,27,72] and other health conditions such as impaired cognitive function [7,73–76], depression [77–81], aggressive behaviors [82] and bipolar disease [83]. The Omega-3 Index was the first omega-3 status test to achieve widespread use in clinical medicine in the US. The appeal of this chronic marker of EPA + DHA status to healthcare providers derives, in part, from its similarity to a more familiar test – hemoglobin A1c which is used as a chronic marker of glycemic status (that is also measured in red blood cells and expressed as a percent). From a technical point of view, RBC FA content is easier to measure in the laboratory than is plasma phospholipid FA content which requires an extra step to isolate the phospholipid fraction. RBCs are already virtually pure phospholipid samples. For all of these reasons, the Omega-3 Index has much to recommend it as the successor to the n-6:n-3 ratio.

5. Conclusion

In the 1980s the confluence of several new discoveries in PUFA biology suggested that the ratio of the “healthy” n-3 PUFAs EPA and DHA to the “inflammatory” n-6 PUFAs would be a sensitive metric for evaluating overall PUFA status in an individual. Now, well into the 21st century, science has advanced substantially in this field in ways that make the ratio obsolete. The bulk of the evidence now supports the CV health benefits of both the n-3 and n-6 PUFAs, and conceptual concerns about the ratio have become clear. It is time to move beyond the n-6:n-3 ratio to biomarkers of long-chain n-3 PUFA status that are more focused, understandable, rational, and actionable. The Omega-3 Index, a metric born in 2004 that fulfills many of the criteria of a *bona fide* risk factor [84], may be the marker of choice for the 21st century.

Conflict of interest

WSH is the President of OmegaQuant, LLC, a laboratory that offers the Omega-3 Index test commercially.



Top: O3I = 0.2608 (n3 HUFA ratio) – 0.0143, r=0.96; Middle: O3I = -0.026 ln(AA/EPA) + 0.142, r=0.84;

Bottom: O3I = 0.1927 $(n\text{-}6:n\text{-}3)^{-0.918}$, r=0.96

Fig. 3. Correlations between the Omega-3 Index and other RBC-based metrics (n = 2312).

Table 3
Hazard ratios for total mortality over 15 years as a function of RBC FA levels [11].

RBC fatty acid	Hazard ratio[a]	*P*-value
Omega-3 Index (EPA + DHA)	0.92	0.0015
ALA n3	0.89	0.37
LA n6	0.99	0.83
AA n6	1.00	0.90
N6:N3 ratio	1.10	0.0021

[a] Multivariable model; HR per 1 SD increase in FA metric.

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