

# CHANGES IN WHOLE BLOOD FATTY ACIDS LEVELS FOLLOWING WHOLE FOOD OMEGA-3 TUNA SUPPLEMENTATION

METAMETRIX INSTITUTE

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***“The National Cholesterol Education Program even launched a “know your number” campaign. But who knows their levels of omega-3s? Well, we should.”***

– Bernadine Healy M.D. *U.S. News and World Report*, August 7, 2008

## INTRODUCTION

The ratio of n-6 to n-3 fatty acids in the modern diet has increased 10-fold to 20:1, leading to a cellular imbalance in downstream chemical products that is having a substantial effect on overall health. Arachidonic Acid (AA) and Eicosapentaenoic Acid (EPA) are the primary precursors of different classes of pro-inflammatory and anti-inflammatory eicosanoids, respectively, whose biological activities have been evoked to justify risks and benefits of polyunsaturated fatty acid (PUFA) consumption.

Some studies have looked at the AA/EPA ratio while others have focused on the total EPA+DHA (Docosahexaenoic Acid) level. Research has found that increasing EPA+DHA and decreasing the AA/EPA ratio results in the production of anti-inflammatory compounds with reduced pro-aggregatory and vasoconstrictive properties. The EPA+DHA level has been associated with health benefits for many conditions, including heart disease, depression, learning disabilities, diabetes, and skin conditions. Eating fatty fish can increase the EPA+DHA level and lower the AA/EPA ratio. Refined or specially filtered fish oil supplements with specified doses of EPA+DHA can make for a more certain prescription, and alleviate concerns about fish being contaminated with mercury or PCBs. Due to significant

individual variation and the increased risk of lipid peroxidation, fish oil supplementation is best implemented following individual laboratory assessments of fatty acids, specifically the EPA+DHA level and the AA/EPA ratio.

## AA/EPA RATIO

The AA/EA ratio is primarily a marker of inflammation.<sup>1</sup> AA serves as substrate for the cyclooxygenase and lipoxygenase enzymes, leading to the production of the 2-series prostanoids and the 4-series leukotrienes, exerting a potent proinflammatory effect. Several clinical intervention studies, as well as cellular and animal models have shown that decreasing the AA, or the AA/EPA ratio results in increased protection against degenerative diseases such as cardiovascular disease, cancer, inflammatory and autoimmune diseases, ADHA, and depression.<sup>2-4</sup> Due to the obvious financial incentive many drugs have been developed to deal with the downstream consequences of this fatty acid imbalance, and the message of increasing n-3's and balancing the fatty acid ratio is not reaching the public. An entire generation of anti-inflammatory drugs, the COX-inhibitors, is based on compounds that block the cyclooxygenase system. Side effects are generated by the overriding of normal cellular controls for regulating the pathways either up or down in specific tissues to generate appropriate local responses.



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Diet and supplementation can both have a significant impact on the ratio. AA provided in the diet is incorporated in membrane phospholipids and enhances eicosanoid biosynthesis; diets high in meats are rich in AA, especially due to the prevalence of corn and corn oil products in feed for cattle and hogs, and can increase the AA/EPA ratio.<sup>5-6</sup> Diets high in EPA and DHA will decrease the AA/EPA ratio. Research has also found that increasing the intake of DHA can lower the AA/EPA ratio, either due to retro conversion of DHA to EPA or a slowed conversion of EPA to DHA. An elevated AA/EPA identifies a patient whose diet is inadequately balanced or who has impaired digestion or endogenous fatty acid metabolism.<sup>7</sup> Controversy exists as to the levels of AA in food and in the diet. The AA content in tuna was found to be half the value noted in the Agriculture Handbook No. 8, making tuna a good source of EPA and DHA, with low AA levels.<sup>8</sup>

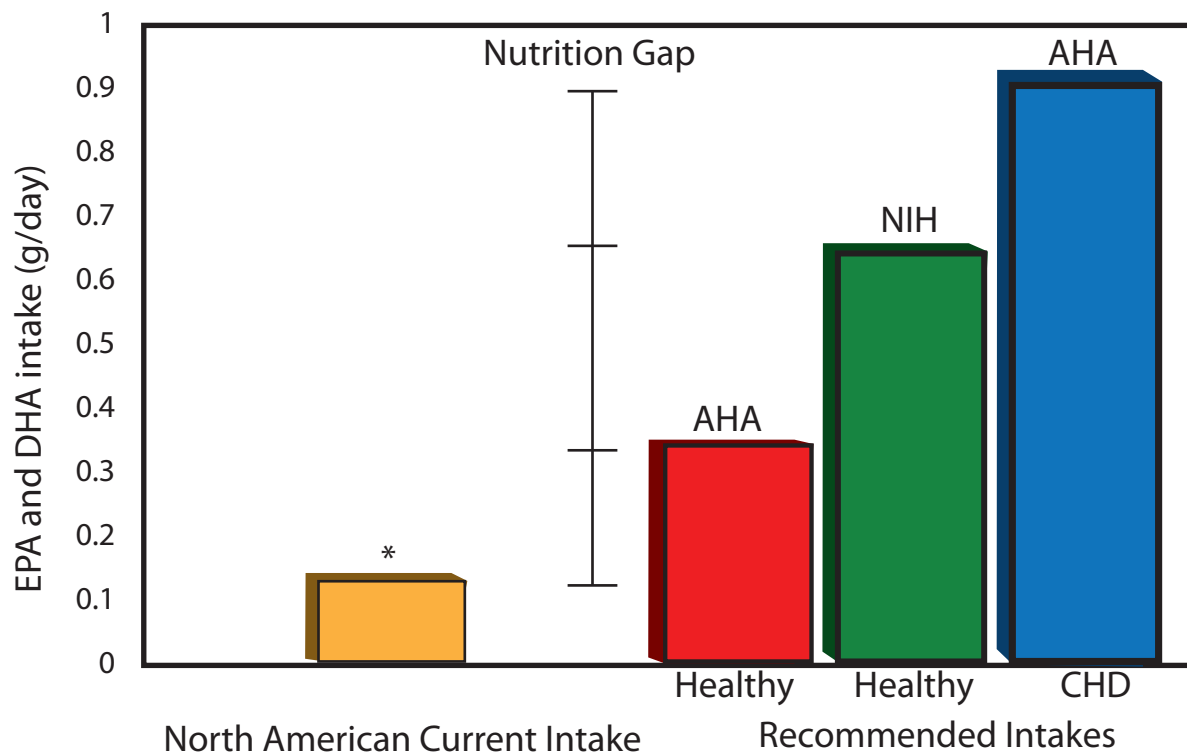
Simply supplementing with fish oils, though, may not offer the desired benefit. Excess supplementation can produce an n-3 fatty acid dominance state, resulting in an extremely low AA/EPA ratio. It is detected as high absolute concentrations of the n-3 fatty acid class members with lowered AA. Omega-3 fatty acid dominance can result in an increased risk of oxidative damage and elevated serum lipid peroxides, which have been shown to be elevated in patients with heart disease.<sup>9</sup> High dose supplementation with fish oils has been shown to increase serum lipid peroxides, rising in proportion to serum PUFA concentrations.<sup>10-11</sup> Excessive n-3 fatty acids can also suppress immune function, leading to infections and poor wound healing. Thus laboratory testing can give guidance in adjusting diet or supplementation to ensure the right balance is achieved.

## BENEFITS OF OMEGA-3 FATTY ACIDS

Unlike AA which leads down a pro-inflammatory cascade, EPA converts to the anti-inflammatory 3-series prostaglandins, and 5-series leukotrienes. Significant improvements were noted in ADHD, Autism, developmental coordination disorder, learning disabilities, depression, bipolar disorder, diabetes, and skin conditions with supplementation of EPA and DHA.<sup>12-25</sup> In large epidemiologic studies EPA and DHA fatty acid levels were associated with significant reductions in total mortality, CHD mortality, cardiovascular mortality, and sudden death, even in those patients with no history of cardiac

disease.<sup>1, 26-30</sup> Since both EPA and DHA are critical for vascular system function, a single value that represents the sum of both fatty acids has been proposed as a useful marker for coronary heart disease, the Omega-3 Index.<sup>29-31</sup> Studies show the Omega-3 Index to correlate with cardiac n-3 tissue levels.<sup>32</sup> When results were expressed as a percentage of the total RBC fatty acids, subjects with a sum of EPA+DHA above 8% had a 90% risk reduction in sudden cardiac death compared to lower values of the sum.<sup>27, 31, 33</sup> The Physicians' Health Study evaluated a variety of CHD risk factors and their association to the development of future sudden cardiac death (SCD); risk was divided into quartiles. The risk factor with the greatest association for avoiding SCD was the Omega-3 Index. The index has a clear dose-response relationship.<sup>27, 34</sup> Research has found RBC EPA+DHA levels to correlate closely with whole blood levels of EPA+DHA with an r-value of .89, and with dried whole blood on filter paper with an r-value of .87.<sup>35</sup>

The NIH recommends a daily intake of 650 mg of EPA+DHA for healthy individuals. The American Heart Association recommends two fatty fish servings per week for healthy people, giving an equivalency of EPA+DHA of 250-300 mg/day, and 900 mg/day for individuals with coronary heart disease. Currently, the total n-3 fatty acid intake in the U.S. is 1.6 g/d, the majority of which is alpha linolenic acid (ALA), with only 0.1 to 0.2 g/d coming from EPA+DHA.<sup>36-37</sup> Figure 1 represents the current intake compared to recommended levels. Such a low intake of n-3 fatty acids has large effects on fatty acid metabolism, the EPA+DHA level, and the ability to lower the AA/EPA ratio.



\* Denomme, Stark, and Holub. *J Nutr.*, 135: 206-211, 2005

FIGURE 1.

### NEED FOR LABORATORY TESTING

While recommendations are helpful in highlighting the essential need of EPA and DHA, they are population based. Although there is a strong correlation between supplementation and the EPA+DHA level and AA/EPA ratio, it is not always predictable due to variations in individual biochemistry. To fully assure an individual has attained a therapeutic level laboratory testing and personalized supplementation doses are needed. The individual variability to supplementation can be due to many factors including: cholesterol level; genes; age; ability to breakdown and digest fats, and level of needed nutrients.<sup>35</sup> Women of childbearing age are better at generating EPA and DHA from ALA. Repletion of a severely depleted patient may also require long-term supplementation. Beyond individual biochemistry there are other concerns in attempting to increase n-3 levels. Some sources of n-3s such as ALA in flax do not readily convert to EPA or DHA. Thus the benefits of EPA+DHA supplementation or dietary changes can really only be evaluated via laboratory assessment. Plasma levels should

show increasing n-3 fatty acid concentrations within 60 days and erythrocyte or whole-blood follow-up testing is best done after 90 days.

### METHODS:

Thirty individuals were initially screened using the Metamatrix Bloodspot<sup>SM</sup> Fatty Acids (BSFA) Profile to determine their levels of n-3 and n-6 fatty acids, and their need for supplementation. BSFA testing measures n-3 and n-6 fatty acids from a simple finger stick and calculates key indicators to establish individual optimal balance. The test specifically measures the n-3 fatty acids ALA, EPA, DHA; n-6 fatty acids Linoleic Acid (LA), Gamma Linolenic Acid (GLA), Dihomogamma Linolenic Acid (DGLA), and AA. Individuals who were low in EPA, DHA, or both were given 2.4 gms of whole tuna oil in 2 divided doses with meals for 90 days, providing 600 mg of DHA and 120 mg of EPA daily. The supplement was a “whole food” type of fish oil derived from tuna. Minimum compliance was set at 80% and determined by pill count. Patients were asked not to alter their current consumption of n-3 containing foods

such as flax, fortified foods or fish but were not required to keep a food diary. Patients were also asked to retest within 10 days of the end of their supplementation period.

**RESULTS:**

Values for the AA/EPA ratio and the EPA+DHA Index were reported as quintile levels. Quintiles for all results were based on the levels reported for each patient’s individual laboratory reports. Results for the AA/EPA ratio are reported as mean quintile levels for the AA/EPA ratio per pre-treatment and post-treatment tests (Table 1) and change in AA/EPA quintile levels per individuals (Figure 2).

The pre-treatment AA/EPA ratio quintile mean was 3.9 (Table 1), a value in the top third quintile. The post-treatment AA/EPA quintile mean was 2.4, representing a mean decline of 1.5 to the mid-second quintile. Paired t-tests of the pre- and post-treatment AA/EPA ratio quintile levels were found to be significant with a p-value of 0.0001.

Table 1.					
EPA+DHA Index			AA/EPA Ratio		
Pre-Tx	Post-Tx	Δ	Pre-Tx	Post-Tx	Δ
MEAN					
1.4	2.8	1.4	3.9	2.4	1.5

In Figure 2 the pre- and post-treatment AA/EPA quintile levels are listed per individual. As reflected by the overall decrease in the mean, the majority of those supplementing with whole tuna oil showed a decrease in their AA/EPA ratio (n=13), while several participants had no change in their quintile level (n=5). No participants had an increase.

*See Figure 2 (page 5)*

Results for the EPA+DHA Index quintile levels are reported as mean quintile levels in pre- and post-treatment tests (Table 1) and change in EPA+DHA Index quintile levels per individuals (Figure 3).

The mean pre-treatment EPA+DHA Index quintile level was 1.4 (Table 1), a value in the first quintile. The post-treatment EPA+DHA Index quintile mean was 2.8, representing a mean increase of 1.4 to the top of

the second quintile. Paired t-tests of the pre- and post-treatment EPA+DHA Index quintile levels were found to be significant with a p-value of 0.0008.

In Figure 3, the pre- and post-treatment EPA+DHA Index quintile levels are listed per individual. The majority of those supplementing with whole tuna oil did have an increase in EPA+DHA (n=14), as reflected by the overall increase in the mean, with half of those (n=7) experiencing an increase of 2 or more quintiles. A minority of participants had no change (n=2), or a decrease (n=2) in their EPA+DHA Index quintile level.

*See Figure 3 (page 5)*

Using a multiple regression equation to evaluate the major contributors to the post-treatment EPA+DHA Index level, post-treatment DHA and post-treatment EPA levels were both found to provide the majority of the increase. Increasing the DHA level made the biggest impact on the post-treatment EPA+DHA Index with a coefficient of r=.74. The post-treatment increase in EPA had a coefficient r=.26. The pre AA/EPA ratio, DHA, EPA, and post-treatment AA/EPA did not make statically significant impacts within the model.

A multiple regression was also used to evaluate the primary contributors of the post-treatment AA/EPA level. The post-treatment EPA level was the only variable that had a significant impact with a coefficient of r=-.82. The majority of the decline in the AA/EPA ratio was due to the post-treatment AA level or the increase in EPA during supplementation.

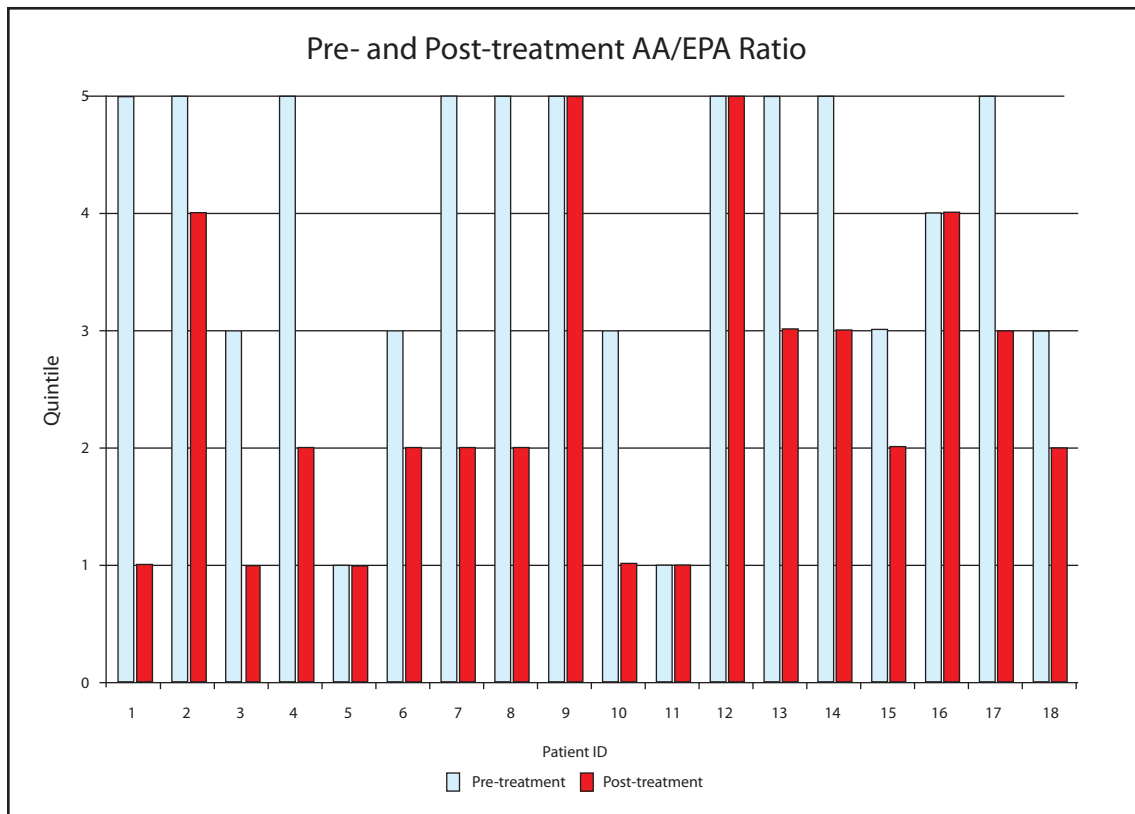


FIGURE 2.

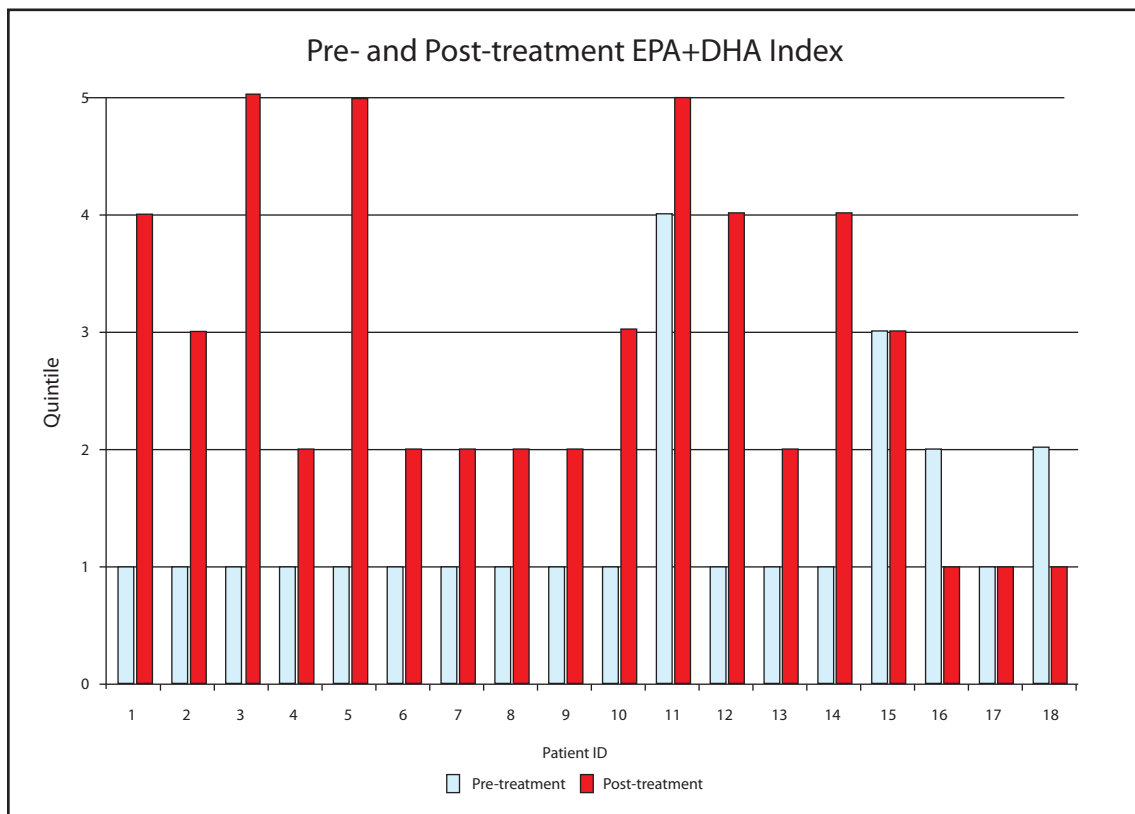


FIGURE 3.

**CONCLUSIONS:**

Though the benefits of fish oil are known, and supplementaton studies have consistently shown to increase the mean EPA+DHA Index and decrease the mean AA/EPA level, the research cannot automatically be transferred to each individual, and for all supplements. This study identifies the benefits of whole food tuna oil preparation, with a naturally occurring high DHA level. The supplemental dose used, 1.6 grams, is less EPA+DHA than has been typically used in past studies, which used 4 – 6 grams of total n-3s. The lower dose was able to increase the EPA+DHA Index and decrease the post-treatment AA/EPA ratio in the majority of post-treatment participants, while also reducing the risk of over saturation and oxidation due to excess n-3 supplementation.

In the current study the mean levels of important fatty acid indicators, such as the AA/EPA ratio and the EPA+DHA Index, made positive changes that were statistically significant. Though every individual did not see a postive change, 10 of the 18 individuals observed started with significantly elevated AA/EPA ratios (5th quintile, as shown in Figure 2). Of those high-risk individuals, 8 of the 10 experienced a significant positive reduction in their AA/EPA ratios. The pre-treatment AA/EPA ratio quintile mean for this group was 5.0, while the post-treatment AA/EPA quintile mean was 3.0, representing a mean decline of 2.0. Of the remaining eight participants, six had less elevated pre-treatment AA/EPA ratios (2nd or 3rd quintiles), with five of the six experiencing post-treatment reductions of one (n=3) or two (n=2) quintile levels. Two participants had pre-treatment levels in the first quintile and remained there through the post-treatment follow up testing. Only 16% (n=3) of the participants who showed any elevation in their pre-treatment AA/EPA ratio (quintile < 1) had no decrease in their AA/EPA ratio.

Fourteen of the eighteen individuals observed started with a low EPA+DHA Index (1st quintile, as shown in Figure 2). Of those individuals, 93% (n=13) experienced a significant positive increase in their EPA+DHA Index, while two experienced no change and two experienced a decrease in their index.

This study follows previously known ideas that supplementing will adjust mean levels, but brings home the point that in order to know if a single patient's level

is adequate and reaches a specific amount you must test that patient's blood level. Testing can identify if, and how well, a patient responds to supplementation. However, it should not be assumed that merely increasing the dose of fatty acids in non-responders is the answer. Issues with the individual's fatty acid absorption, metabolism, and underlying inflammatory triggers could also be involved, necessitating additional, more in-depth fatty acid analysis. The levels and ratios of fatty acids have been shown to make a significant impact on health, making their evaluation critical in any clinical workup.

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