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Technical Report

Choosing the Best Marine-derived Omega-3 Products for Therapeutic Use: An Evaluation of the Evidence

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Choosing the Best Marine-derived Omega-3 Products for Therapeutic Use: An Evaluation of the Evidence

There is overwhelming data to recommend a wide range of therapeutic uses for marine-derived omega-3 fatty acids (primarily EPA and DHA). Likewise, there are also an overwhelming number of different forms, sources and ways to deliver these omega-3 fatty acids; which unfortunately, has led to confusion in product selection for both clinician and patient alike. We will walk through the most common issues discussed in selecting the appropriate omega-3 fatty acid product, with the goal to bring clarity to the decision-making process.

For the most part, the marine omega-3 fatty acid category is dominated by products that can be best described as “fish oil.” That is, while there are products available that deliver omega-3 fatty acids from other marine sources, nearly all the available research has been done with fish oil derived fatty acids. This fish oil data has become the benchmark for efficacy and safety, and is the standard to which we compare throughout this paper.

The following are the main sources of marine omega-3 fatty acids

- **Fish Body Oil:** The largest biomass used to create marine-derived omega-3 fatty acids comes from small oily fish caught in the cold waters off the coast of Chile and Peru. The fish species most commonly used are mackerel, anchovies, and sardines. Concentrations of these purified oils are the most common therapeutic product used in dietary supplements and pharmaceutical products throughout the world. Other species used to produce fish oil may include salmon, tuna, menhaden, herring and other minor species.¹
- **Cod Liver:** As a by-product of the cod meat market, cod liver can be used to provide a blend of fatty acids similar to un-concentrated fish body oil.
- **Krill:** Small crustaceans which feed upon plankton and become feed for many marine mammals, especially whales. Krill is processed by factory ships immediately upon capture in the cold waters off the coast of Antarctica. Krill oil is fairly low in EPA and DHA, but contains small amount of the carotenoid astaxanthin.
- **Calamari:** A recent, but small, player in omega-3 fatty acid industry is calamari or squid oil. This oil has a higher ratio of DHA over EPA than is typical of fish. This oil is a byproduct of the calamari food industry.
- **Mussels:** Shellfish are a very minor source of commercially available omega-3 fatty acids. Nonetheless, several products are currently available from the fatty acids derived from Green-Lipped Mussels (*Perna canaliculus*). The diverse fatty acid profile of these mussels includes EPA and DHA (in a ratio of about 65:35). Limited research has been done using products derived from mussels for traditional omega-3 related outcomes (mostly arthritis and inflammation studies).
- **Algae:** Various species of algae are commercial sources for omega-3 fatty acids. These products are almost exclusively DHA. Most of the pure DHA raw materials, especially pure DHA used for addition to infant formula, comes from these algal sources.

¹ For detailed information about status and environmental performance of fisheries worldwide (fish species, harvest statistics, regulations and sustainability) see the website: www.fishsource.com

Additional considerations on marine omega-3 sourcing:

Kosher: Only fish or algae sources are products that can be deemed truly “kosher,” however; additional manufacturing processes may influence the ability to officially label a finished product (i.e. soft gelatin capsule) with a particular kosher certificate.

Vegetarian/Vegan: While many vegetarians choose to consume fish oil products even if they avoid consuming fish, strict vegans will avoid all marine lipids with the exception of algal sourced products. Since EPA can be formed by consuming either DHA from algae or alpha-linolenic acid from flax seed oil, these may be suitable options for strict vegan individuals. It should be noted that while these vegan omega-3 source are likely to increase blood levels of EPA and DHA, there are no data yet to suggest that these alternatives will have the same risk-lowering benefits as EPA & DHA from fish.

Gluten: Marine fatty acids are gluten-free and softgel manufacturing should not introduce gluten to finished products.

GMO-status: None of the biomass (fish, krill, squid or algae) used to produce commercially available fatty acids *today* are known to be genetically modified. Under most global definitions, this would make these products “GMO-Free.” Some groups (e.g. GMO Verified Project) have proposed very strict definitions of GM status whereby the inability to verify the GM status of foods eaten by harvested fish in the wild (or the GM status of the animals which contribute the gelatin for making softgels) would thereby not permit a GMO-Free status. Genetic modification of certain algae for the production (through fermentation) of EPA and DHA is in the works and may soon become commercially available. Also, genetically-modified plants (soy, canola etc.) are also being researched as non-marine sources of EPA and DHA.

Sustainability Issues: One of the concerns with using large quantities of marine omega-3 fatty acids for therapeutic use is the long-term sustainability of harvesting the needed biomass. The debate over which source(s) might be in danger of overharvesting or are being harvested ecologically is quite controversial and is made more difficult by the fact that no single final authority defines “sustainability” for the global community. Fisheries that supply both fish meal and fish oil are managed by a number of regulatory bodies around the world where harvest limits are set for fish species, fishing seasons and fishery zones. Obviously there is a seasonal variability in the biomass which is controlled by both local and global ocean conditions, affecting the year-to-year availability of EPA and DHA.

Controversy over krill sustainability appears to be more in debate. Most notably, in 2010 the retailer Whole Foods declared they would not sell krill products because of data they believed linked krill harvesting with reduced levels of animals that depend on krill for food. Since that time, the Marine Stewardship Council and other organizations have approved several of the largest krill harvesting companies as being “sustainable.” Whole Foods still, as of 2013, does not sell krill products.

The future of the sustainability of the marine biomass is influenced by global ocean fluctuations and the growing need for EPA and DHA. Tension between sustainability on the one hand and the use of more sustainable GMO-produced EPA and DHA from plants on the other hand, will no doubt increase the controversy.

Allergies to fish and shellfish related to omega-3 products

Since the changes required for food allergen labeling went into effect in the US in 2006, there is some confusion as to how fish oil products should be labeled and whether individuals allergic to fish can safely consume fish oil. The eight allergens that require mandatory labeling includes both fish and shellfish (also soy, wheat, eggs, peanuts, tree nuts, and milk). However, the labeling requirements exempt the need to label ingredients which are highly refined oils containing no allergenic proteins. Highly-refined fish oils (like certain soybean oils), therefore do not need to be listed in a separate “contains the following allergens” statement on the label. Some companies using highly-refined fish oils still choose to include “Fish” in a list of allergens, primarily from a product liability standpoint. Nonetheless, the supplement facts box or front panel of a fish oil product must still declare that the ingredient itself is concentrated fish oil, thereby notifying users that the content contains fish-derived oils.

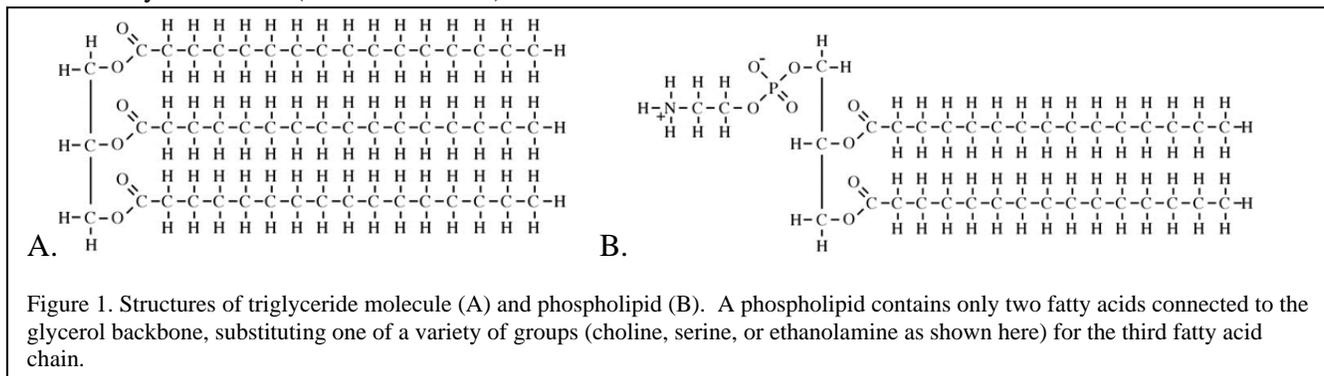
Labeling issues aside, what is the likelihood that individuals who have known allergies to finned fish might also have an allergic reaction to a fish oil product? The answer appears to be: extremely unlikely. First, allergic reactions to fish are well understood and identified to very specific proteins. Highly refined fish oil products are virtually absent from any detectable protein and there is no known allergens to fish-derived fatty acids or even to fish-derived gelatin used in some capsules. This notion was actually tested in a small-scale study where individuals with known allergies to finned fish were given fish oil supplements to see how they would react.² Two different fish oil supplements were tested in 6 subjects with known fish allergies by both skin and oral challenges. None of the subjects reacted in any way to either product.

These data, while limited, do agree with the notion that highly-refined fish oil products contain no reactive allergens and should be safe to consume by individuals with mild-to-moderate fish allergies. Highly sensitive individuals or those with life-threatening fish allergies should probably avoid the use of fish oil products out of the abundance of caution, and look to get omega-3 fatty acids from plant sources such as algae (DHA) and flax (ALA). We are not aware of any published articles that similarly test krill or mussel-derived fatty acid products (for shellfish allergies).

² Mark BJ, Beaty AD, Slavin RG. Are fish oil supplements safe in finned fish-allergic patients? *Allergy Asthma Proc.* 2008 Sep-Oct;29(5):528-9.

Fatty Acid forms and structures

When fatty acids are harvested from their source, they are typically in the form of triglycerides (TG), phospholipids (PL) or free fatty acids (FFA). When these fatty acids are delivered as dietary supplements or pharmaceutical products they can be delivered as triglycerides (in a purified but un-concentrated form or a concentrated re-esterified form [rTG]), as ethyl-esters (EE), as free fatty acids (FFA), or as phospholipids. These differences may alter the bioavailability and, perhaps, the efficacy of the fatty acids used (discussed below).



The processes that transform the “crude oil” harvested from fish into the 3 main fish oil products for therapeutic use involves a number of specific steps.³ We will briefly describe each:

1. Deodorization: This process takes the crude oil from the fish and by use of an evaporator, removes the free fatty acids and many of the organic pollutants which may be in the oil. This process is sometimes referred to as molecular distillation since heating and cooling is used in this process. Some steam deodorization is still used by some companies.
2. Ethylation: This process removes the fatty acids from the glycerol back bone and after neutralizing with a dilute acid, forms ethyl ester fatty acids. Essentially a free fatty acid with a ethanol attached to the carboxyl end.
3. Distillation/concentration: Under low vacuum and heat, this molecular distillation removes shorter chain ethyl esters and saturated fatty acids and can be continued until the desired EPA & DHA content is achieved (within limits- see CO₂ concentration below⁴)
4. Cold Filtration: This process precipitates solids that cloud the oil. These precipitates are filtered and removed. This step is sometimes called winterization.
5. Glycerolysis: This process allows for the enzymatic (or acid/base catalyzed) reattachment (re-esterification) of the concentrated fatty acids (mostly EPA and DHA) molecules to a glycerol molecule creating triglyceride and diglyceride molecules. These molecules might be deemed “bio-identical”, as they are chemically the same as the original TG compounds, only with 2 or 3 times the number of EPA or DHA molecules attached to the glycerol backbone. This process is obviously not performed when products will be sold as ethyl esters.
6. Molecular Distillation: Similar to the first deodorization/distillation, this process removes excess glycerol or fatty acids not reacted in the previous step.
7. Clay Filtering (Bleaching): This is a step to remove very small contaminants which can be bound and removed by a special clay mixture.
8. Blending: The resultant oil is mixed with agents to protect oxidation (e.g. vitamin E, antioxidant oils etc.) and blended with other similar oils as necessary to meet specific total omega-3 fatty acid or EPA/DHA specifications, before being put in drums for shipping.

³ Krill biomass requires a multi-step process involving solvent extraction and various filtration steps which differ from fish oil production.

⁴ CO₂ “Super-critical” distillation: Some companies add an additional step using a special system which allows for super critical distillation using CO₂. The oils used for such processes have usually gone through most or all of the steps above. The additional distillation process allows for a higher concentration of EPA and DHA (especially when using EE forms).

Bioavailability Studies:

The efficacy of omega-3 fatty acid therapy is affected by their bioavailability.⁵ Therefore, numerous studies have been performed to compare short and long-term bioavailability in human subjects using omega-3 fatty acids from different sources and in different molecular forms. We will first discuss the studies using fish oil preparations, and then address the question of krill vs. fish oil.⁶

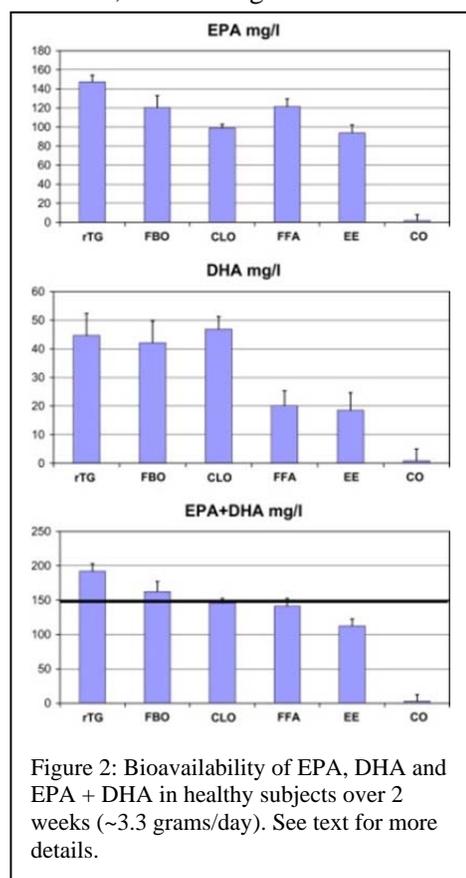
Ethyl Esters vs. Triglycerides

Since the creation of the ethyl ester (EE) forms of omega-3 fatty acids, many people have questioned the potential change in bioavailability of these forms, compared to the natural triglyceride forms. The early studies were small, but already these data revealed either a slightly reduced bioavailability of the EE forms (compared to TG forms) in the absence of additional dietary fat or a statistically similar bioavailability between EE and TG forms. However, several larger and better designed studies have shown a superior bioavailability of the rTG forms over EE forms.

In fact, one of the better studies performed to date compared similar doses of EPA and DHA using 5 different forms: un-concentrated triglycerides (what they called fish body oil-FBO), Cod Liver Oil (similar TG form as FBO), rTG, EE, or FFA, along with a “placebo” of corn oil (CO). In this study, 72 subjects were randomly assigned 3.3 grams per day of a blend of EPA + DHA daily as capsules for 2 weeks.⁷ Serum fatty acids (which combined serum TG, PL and cholesterol esters) were analyzed at baseline and after 2 weeks. Figure 2 shows the changes from baseline to 2 weeks in EPA, DHA and EPA+DHA in subjects consuming these different forms. In these subjects, the bioavailability of EPA+DHA from re-esterified triglycerides (rTG) was superior (+24%) when compared with natural fish oil (FBO+CLO), whereas the bioavailability from ethyl esters (EE) was inferior (-27%) to the natural TG and nearly 70% less bioavailable than the rTG in these subjects. The authors suggest that the increased bioavailability of rTG over the un-concentrated TG form may be due to the fact that rTG products also contain di-glycerides along with a very small amount of mono-glycerides which act as “partially digested forms” of the natural triglyceride, potentially enhancing the bioavailability over the natural fish body oil. Concerning the EE form, numerous studies have shown a decreased lipase enzymatic activity when ethyl ester substrates are used,

perhaps accounting for their decreased absorption when consumed away from a meal containing fat.

Ultimately, what we would like to know is whether any differences in bioavailability over two weeks might translate into long-term differences in fatty acid incorporation into important tissues and



⁵ Several studies suggest that fatty acids may act within the intestinal lumen, prior to becoming available in the bloodstream, in which case efficacy would not require absorption. In addition, individuals with limitations for fat absorption will clearly show differences in their ability to benefit from omega-3 fatty acid therapy. These nuances are beyond the scope of this particular overview.

⁶ For a recent review of EPA and DHA bioavailability studies see: Schuchardt JP and Hahn A. Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids*. 2013 Jul;89(1):1-8.

⁷ Dyerberg J, Madsen P, Møller JM, Aardestrup I, Schmidt EB. Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins Leukot Essent Fatty Acids*. 2010 Sep;83(3):137-41.

whether these differences can be measured in a clinically meaningful outcome. These sorts of studies have actually been carried out by researchers in Germany, where they looked at the incorporation of EPA and DHA into red blood cell membranes, commonly referred to as the omega-3 index, when individuals consumed either EE or rTG forms of fish oil.

This study looked at 150 hyper-lipidemic subjects who were also taking statin drugs.⁸ Subjects were given soft gelatin capsules containing EPA (1008 mg) and DHA (672 mg) daily in either rTG or EE forms (corn oil used in placebo group); and subjects were followed for 6 months. Figure 3 shows the change in omega-3 index (%EPA+DHA in RBC plasma membrane). Subjects consuming the rTG form had, on average, a statistically higher omega-3 index than those consuming the EE form after three months, which was maintained even after 6 months of daily intake.

In a separate publication, the lipid lowering effects of these two therapies were discussed.⁹ What they found was that while both the EE and rTG reduced serum TG levels in these patients compared to placebo; the rTG changes were nearly double that of the EE form and the only therapy to reach statistical significance was the rTG therapy (Figure 4).

Hypertriglyceridemia is quite prevalent across a wide-range of subjects in the United States, especially in those prescribed statin drugs. The use of a similar dose of concentrated omega-3 fatty acids as rTG provided as a dietary supplement (the only pharmaceutical products currently available are EE) can easily be obtained in 2 softgel capsules. It is our view that when available, the “bio-identical” rTG concentrates are preferable to EE forms for clinical therapy and provide much more “payload” of EPA and DHA when compared to un-concentrated fish oil triglycerides.

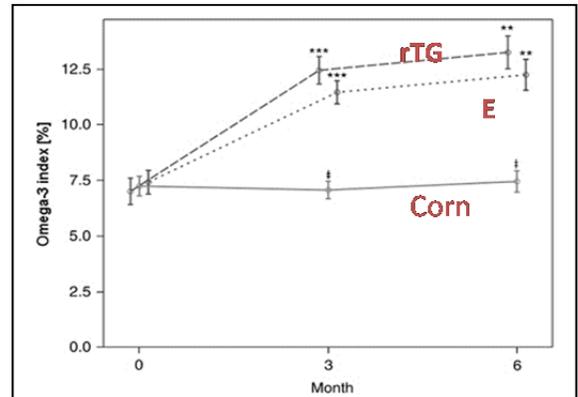


Figure 3: Change in Omega-3 index in hyperlipidemic subjects consuming 1680 mg of EPA + DHA over a 6 month period of rTG or EE fish oil.

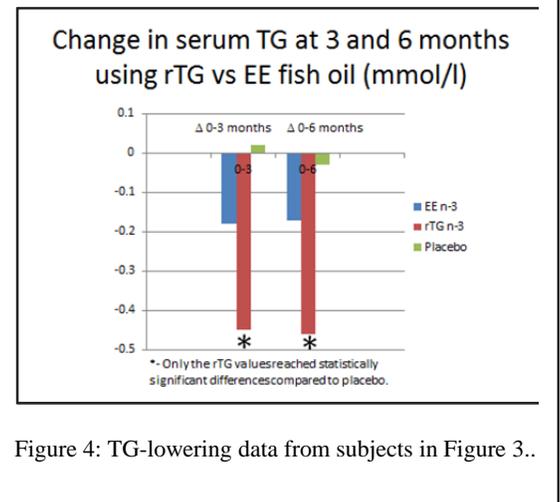


Figure 4: TG-lowering data from subjects in Figure 3..

⁸ Neubronner J, Schuchardt JP, Kressel G, Merkel M, von Schacky C, Hahn A. Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters. *Eur J Clin Nutr.* 2011 Feb;65(2):247-54.

⁹ Schuchardt JP, Neubronner J, Kressel G, Merkel M, von Schacky C, Hahn A. Moderate doses of EPA and DHA from re-esterified triacylglycerols but not from ethyl-esters lower fasting serum triacylglycerols in statin-treated dyslipidemic subjects: Results from a six month randomized controlled trial. *Prostaglandins Leukot Essent Fatty Acids.* 2011 Dec;85(6):381-6.

Bioavailability: Krill Oil vs. Fish Oil

In the past decade, the market has been flooded with information about the use of, and purported superiority of, omega-3 fatty acids from krill. These claims have primarily come from two properties of krill oil: that it is derived mostly of phospholipids (PL-as opposed to TG) and that it contains trace levels of astaxanthin--a bioactive carotenoid. Additionally, some studies have suggested that these properties, particularly the PL nature of the fatty acids, account for a superior bioavailability compared to fish oil. We will examine this claim first.

As of this writing, only short-term and very limited comparisons are available to ascertain the relative bioavailability of krill oil vs. fish oil. One group studied the difference between the use of krill oil and menhaden oil (fish body oil- natural TG) or placebo (olive oil) in their ability to alter plasma fatty acids when consumed by overweight and obese subjects (n=76).¹⁰ Each subject was to consume 2g/day of each oil for 4 weeks before being tested for changes in plasma fatty acid levels. It is important to note that the 2 grams of menhaden oil contained 212 mg of EPA and 178 mg of DHA (390 mg total), while the krill oil preparation contained 216 mg EPA and 90 mg of DHA (306 mg total). Compared to olive oil, both the krill and menhaden oil significantly increased the EPA and DHA levels of the subjects; Krill- EPA (+ 89%), DHA (+23 %), Menhaden- EPA (+ 81%), DHA (+ 45%). These data suggest that the bioavailability of EPA and DHA from krill and un-concentrated menhaden oil are statistically similar. In this study, the systolic blood pressure response in the control group differed significantly from that in the menhaden group (P = .032), but no significant differences were present for krill. This is likely due to the fact that menhaden oil provides a much higher DHA content (most of fish oil's antihypertensive activity seems to be due to DHA levels).

The second study often cited was a 7-week study comparing the change in plasma fatty acids in subjects with "normal or slightly elevated" lipids when given either krill or fish oil.¹¹ This study compared 6 capsules of krill, providing 543 mg of EPA+DHA or 3 capsules of fish oil (unspecified form) providing 864 mg of EPA+DHA. Compared to control subjects (un-supplemented subjects) both krill and fish oil were able to statistically increase EPA and DHA in those consuming each. However, while the average increase in EPA and DHA was slightly higher in the fish oil group, the difference between the groups was not statistically significant. This has led the authors (and many krill advocates) to suggest that this data is proof that a lower dose of krill is equivalent to a higher dose of fish oil. A closer examination of the data show that the standard deviations of the fatty acid levels at both time points (baseline and 7 weeks) is so large, that no real interpretation of these data is possible. Even so, their data did show a statistically significant drop in arachidonic acid in the fish oil group while showing a statistically significant increase in AA in the krill group. The clinical significance of this change in arachidonic acid, generally favoring the fish oil group, is unknown. Regardless, we find it curious that the authors designed the study with such a discrepancy in EPA+DHA, since they could have easily given 2 (instead of 3) fish oil capsules to get a near equivalent dosing comparison to study.

Finally, we turn to the only study which compares equivalent doses of EPA+DHA from krill, rTG and EE fish oils.¹² Unfortunately this was a single dose, 72 hour study which measured changes only in plasma phospholipids, unlike the previous krill studies (plasma fatty acids) or the long-term fish oil study (omega-3 index/RBC FA). Twelve healthy males were recruited to consume each of the three omega-3

¹⁰ Maki KC and Reeves MS et al. Krill oil supplementation increases plasma concentrations of eicosapentaenoic and docosahexaenoic acids in overweight and obese men and women. *Nutr Res.* 2009 Sep;29(9):609-15.

¹¹ Ulven SM, Kirkhus B, Lamglait A, Basu S, Elind E, Haider T, Berge K, Vik H, Pedersen JI. Metabolic effects of krill oil are essentially similar to those of fish oil but at lower dose of EPA and DHA, in healthy volunteers. *Lipids.* 2011 Jan;46(1):37-46.

¹² Schuchardt JP, Schneider I, Meyer H, Neubronner J, von Schacky C, Hahn A. Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations--a comparative bioavailability study of fish oil vs. krill oil. *Lipids Health Dis.* 2011 Aug 22;10:145.

preparations (cross-over design- 14 days apart) containing a total of 1680 mg of EPA+DHA. The particular products used required 4 capsules of fish oil (rTG or EE) or 14 krill oil capsules to obtain the necessary EPA+DHA. Blood samples were taken before dosing (7 AM) and at 2, 4, 6, 8, 24, 48, and 72 h after the intake of the capsules.

Like the previous study, due to high standard deviation values, there were no significant differences for EPA, DHA or the sum of EPA+DHA levels between the three treatments. The authors did note non-statistical “trends” in higher levels of plasma PL-EPA when subjects consumed krill. When one examines the data closely it does appear that this trend may indeed indicate a higher plasma phospholipid level in individuals consuming equal levels of EPA+DHA from krill vs. fish. The conclusion, however, that krill is a “better” source of EPA+DHA is complicated by at least three issues. The first, obviously, is that these non-statistical trends were performed with a single dose in only 12 male subjects; this was a pilot study at best. Secondly, this study measured plasma phospholipids, not omega-3 index of total plasma fatty acids. This is important because it is known that PL can be incorporated into chylomicrons directly and since krill provides most of its fatty acids as PL (they reported that about 22% of the krill oil was FFA), the fish oil fatty acids may have been incorporated in TG, cholesterol esters or FFA. In fact, the “trend” in the increase of plasma PL could have been a 72 hour artifact of consuming krill PL over fish rTG (EE forms performed worse than both).

Lastly, and perhaps most important for practical clinical consideration, it took 14 krill oil capsules to provide 1680 mg of EPA+DHA; something that can now be provided easily in two concentrated fish oil capsules (they used 4). The most widely commercially available krill oil products typically contain only 90-120 mg of EPA+DHA per capsule; while even un-concentrated fish oil products contain 300 mg of EPA+DHA per capsule. Even so, while provided only 1/3 of the amounts of EPA+DHA, krill oil products are typically 5-10 times more expensive. The economic comparisons to concentrated rTG products, which can obviously provide 7-8 times more EPA+DHA per capsule, are similar. Unless a higher amount of EPA+DHA can be delivered by krill for a much more economical price, fatty acids from krill would need to be at least five times (perhaps even 7-10 times) more bioavailable than fish TG to be considered either therapeutically or economically equivalent. That said, there is no reason to believe that similar doses of EPA+DHA from krill would have an inferior biological effect than those proven in studies using fish oil-derived EPA+DHA.

Astaxanthin from Krill

Astaxanthin is a reddish-colored xanthophyll (carotenoid -similar to the compound zeaxanthin) that is found in a variety of marine organisms, from algae to salmon. Krill biomass contains about 120 ppm astaxanthin and most krill oil preparations claim a small amount of it on their label. To date, no studies have been done using krill oil-derived astaxanthin, making the various marketing claims difficult to evaluate. Microalgae sourced (and some synthetic sources) are the commercially available forms used in the limited clinical studies using astaxanthin. For context, the few studies available using astaxanthin in humans used doses ranging from 4 mg to 20 mg per day; the average krill oil capsule claims to have 0.5-0.8 mg of astaxanthin per capsule.^{13,14} Perhaps additional studies will be performed attempting to understand the role and benefits of delivering astaxanthin from krill oil, but currently there are no such studies.

¹³ Earnest CP, Lupo M, White KM, Church TS Effect of astaxanthin on cycling time trial performance. Int J Sports Med. 2011 Nov;32(11):882-8 Effects of astaxanthin on oxidative stress in overweight and obese adults.

Differential uses of EPA and DHA

We have previously reviewed the differential uses of EPA and DHA in a paper that is available at www.pointinstitute.org. A figure summarizing the evidence-based usage of EPA and/or DHA can be seen below.

Condition-specific recommendations from the data presented.			
Condition	EPA-Rich	EPA/DHA	DHA-Rich
General Health		XX	
Improving w3:w6 ratio		XX	
Low-Risk CVD prevention		XX	X
High CVD Risk/Atherosclerosis		X	XX
Inflammatory Diseases		XX	
Depression	XX	X	
Macular Degeneration			XX
Alzheimer- Dementia		X	XX
Maternal- Childhood Devel.		X	XX

Quality Control Issues of fish oil and related products

We are several decades into the regular use of fish oil derived omega-3 fatty acids as dietary supplements and pharmaceutical products worldwide. Nearly all of the quality control issues that plagued the first few years of fish oil availability, such as heavy metal contamination, pesticide residues and oxidation are rare instances in today's products. A number of highly reputable organizations (e.g.: GOED-Global Organization for EPA and DHA; CRN- The Council of Responsible Nutrition) have developed standards for fish oil products which set specific limits for heavy metal contamination, a wide variety of organic pollutants and oxidation limits. Most of the global fish oil providers maintain all their products to these high standards. This is especially true of the concentrated products (rTG and EE forms); and since heavy metal and pesticide residue are virtually impossible to add during the manufacturing process, monitoring oxidation of the fatty acids is one of the critical steps in producing a high quality product.

Fish oil oxidation is measured using two methods. The first measures oxidized fatty acids directly as a peroxide value (PV or POV). Since these peroxides are transient and can form secondary oxidized molecules (like aldehydes), a second test is used to detect these oxidized compounds--the anisidine (or p-anisidine) test. When we combine these values by adding the aniside value to twice the peroxide value (AV+2PV),- we get the TOTOX value.¹⁵ To control the oxidation of the fish oil raw material and finished product, most manufacturers add a variety of antioxidant compounds. The most popular are vitamin E,

¹⁵ For a more detailed explanation of this, a helpful summary can be found online at: <http://www.oilsfats.org.nz/Oxidation%20101.pdf>

vitamin A, flavonoids, and rosemary extracts or other spice extract; rarely synthetic antioxidants are used. Most commercially available products will contain one or more of these antioxidants, at very low doses, in the finished product. Manufacturers of liquid-filled bottles or softgel capsules also utilize nitrogen (to purge available oxygen), low light and cold temperatures in the manufacturing process to reduce oxidation and extend shelf-life. Products used after their expiration date should be thrown away, as oxidized fish oil can act as a pro-oxidant and limit the benefits realized if consumed.

Enteric Coated Capsules and Flavored oils

In the early days of fish oil therapy it was common to experience unpleasant GI side-effects of consuming fish oil, including upset stomach, changes in bowel consistence and the dreaded fish oil burp. Obviously, some people are more prone to these issues than others and the data from an overwhelming number of clinical trials shows fish oil to be extremely well tolerated in a wide-range of subjects. Even so, to combat some of the unpleasant side-effects of consuming fish oil some manufacturers have taken to adding flavors into some fish oil products or have enteric-coated the softgel delivering the fish oil. Are these necessary and do they alter the efficacy or safety of the products?

For the most part, the small amount of flavoring used to give the oil a hint of citrus or other (mostly fruit) flavors have no real measurable impact on the quality or efficacy of the oil, and they can be made from natural sources. It should be noted that once most flavors are added to the oil, they very often interfere with the anisidine test in the final product which relies on colorimetric analysis. In these cases, the oxidative analysis needs to be done before flavoring is added, while any changes in the peroxide value can be used to monitor the oxidation which may have been introduced between flavoring and bottling or encapsulation. Since many people experience burping independent of the fish oil quality, flavoring the oil may prevent this issue from causing them to discontinue the product, creating a better clinical outcome. Fish oil provided in a liquid form is often flavored to help provide a pleasant aroma and flavor at the point of consumption.

Over the past several years, some fish oil soft gelatin capsules have been provided with various forms of enteric coating, preventing the release of the fish oil in the stomach which virtually eliminates the ability to burp-up any fishy taste. There are two potential issues with this approach that clinicians should be aware of before considering the use of enteric-coated fish oil products: potential for reduced bioavailability and synthetic compounds used to make enteric-coating. The second issue is more straightforward, so we will address that first.

A truly “enteric-coated” product must meet specific criteria for disintegration; allowing it to remain intact in the stomach while still releasing the active ingredients in the small intestines. To do this, capsules must be coated with a series of chemicals to allow for pH-specific performance. The problem is that the chemicals needed to make soft gelatin capsules truly enteric-coated are typically not the sort that healthy consumers and their clinicians want to consume. Things like: methyl acrylate-methacrylic acid copolymers, cellulose acetate succinate, hydroxy propyl methyl cellulose phthalate, and polyvinyl acetate phthalate (PVAP) to name a few. While these chemicals are very capable of creating the ideal gastric/enteric release profile, it is no wonder that most companies are reluctant to label these additional ingredients. In fact, companies that choose to label these ingredients as merely “enteric coating” are not in compliance with the labeling requirements for food or dietary supplements in the US.

There are numerous products which claim to have more natural forms of enteric coating; and while these coatings do avoid most of the chemicals listed above, the precision of their enteric-coating does not meet the same standards as the studied products using the synthetic chemical coatings. Either way, the additional handling and coating increases the cost of the final product and increases the chance for exposure to oxidation. It also adds the potential to diminish bioavailability.

It is well-known that marine fatty acids need to be partially digested with pancreatic lipases in combination with the help of bile salts in the small intestines prior to absorption. These free fatty acids and monoglycerides form mixed micelles and move into enterocytes where they are then packaged into chylomicrons to be carried into the blood stream. The concentration of bile salts and lipase enzymes in the duodenum is intended to interact with fatty acids immediately after they leave the stomach allowing for

absorption within the upper jejunum. However, in the case of enteric-coated products, this may not occur for 30 minutes or more after arriving in the small intestines (USP analysis allows for up to an hour in simulated intestinal fluid).¹⁶This delay may act to reduce the interaction of the ingested contents of the enteric-coated capsule with the bile salts and enzymes, thereby reducing bioavailability.¹⁷

There is, on the other hand, data that suggests that certain coated fish oil capsules have adequate bioavailability. One particular study compared EPA and DHA (rTG form) from either an uncoated soft gelatin capsule or a gastric-acid resistant coated capsule in twelve subjects following a cross-over design.¹⁸ After subjects consumed a single dose (4 capsules containing 1680 mg of EPA+DHA) their plasma PL changes in EPA and DHA were followed for 72 hours. There were no statistical differences between the area under the curve (AUC), the timing (Tmax) or peak concentrations (Cmax) between ingesting the coated and uncoated products. Dissolution information was described for these gastric-coated capsules, showing that they complied with the European Pharmacopeia for gastric-coated soft gelatin capsules. It should be noted that the European Pharmacopeia specifications for this type of coating requires softgels to dissolve (once past the gastric resistance portion) in less than 30 minutes; although this study claims these particular capsules released in less than 15 minutes.

Nearly all other studies using coated capsules (standard enteric-coated) use EPA and DHA delivered as free fatty acids (FFA), which do not require digestion by lipase and bile to prepare them for absorption. In this form, EPA and DHA appear to be bioavailable, at least compared to the poor absorption of EE form (Lovaza) consumed during a low fat meal.¹⁹ It is interesting to note that the overwhelming published data for enteric-coated fish oils has been for direct gastrointestinal use in patients with inflammatory bowel disease (particularly Crohn's disease²⁰) and colon polyps²¹; where a delayed absorption may be helpful. Dietary supplement companies selling enteric-coated TG, rTG, EE or PL forms of EPA and DHA should be asked to provide pharmacokinetic/bioavailability results from their own products (lot specific) and about the quality control procedures they use to ensure batch-to-batch consistency for capsule dissolution. Without this information, it is difficult to ensure that the product will have the same properties as those described in the limited studies described here.

Dietary Supplements vs. Pharmaceutical Products

Currently there are only two approved pharmaceutical products on the market in the US. Lovaza (formerly Omacor: GlaxoSmithKline), an EE fish oil product providing 465 mg of EPA and 375 mg of DHA (840 total) in a single softgel; and Vascepa (Amarin) which is a 1 gram capsule of an EE form of EPA-only; both indicated for severe hypertriglyceridemia (TG>500mg/dl). The rationale for the newer EPA-only Vascepa, according to Amarin's marketing perspective, is that EPA does not raise LDL-C like DHA does. Neglected in this perspective is that DHA also raises HDL-C, and dramatically increases LDL particle size which is both cardio-protective and the very reason LDL-C is increased when DHA is

¹⁶ USP Disintegration and Dissolution of Dietary Supplements-June 2011

http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/revisions/2040disintegrationdissolution.pdf

¹⁷ Lack of proper enteric-coating was a major culprit in the failure of numerous clinical trials involving enteric-coated garlic tablets- used to avoid the taste of garlic. Lawson LD et al. Allicin release under simulated gastrointestinal conditions from garlic powder tablets employed in clinical trials on serum cholesterol. *Planta Med.* 2001; 67(1):13-8

¹⁸ Schneider I, Schuchardt JP, Meyer H and Hahn A. Effect of gastric acid resistant coating of fish oil capsules on intestinal uptake of eicosapentaenoic acid and docosahexaenoic acid. *J. Functional Foods* 2011 3:129-133

¹⁹ 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 Nov-Dec;6(6):573-84.

²⁰ Turner D, Zlotkin SH, Shah PS, Griffiths AM. Omega 3 fatty acids (fish oil) for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev.* 2007 Apr 18;(2):CD006320.

²¹ West NJ and Clark SK, et.al. Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. *Gut.* 2010 Jul;59(7):918-25. doi: 10.1136/gut.2009.200642

administered. This “anomaly” of DHA (slight increase in LDL-C) is actually a signal of a beneficial shift in lipid metabolism. Unfortunately, since the lipid-lowering guidelines are fixated on reducing LDL-C as a primary goal, the marketing of an EPA-only product for cardiovascular health will no doubt mislead many who target LDL-C alone.

Some dietary supplement companies refer to their fish oil products as “Pharmaceutical Grade.” While this term is misleading, in that no such designation exists for products which are not approved as pharmaceuticals in the US, reputable suppliers of highly concentrated fish oil dietary supplements provide products which are just as pure and effective as those approved as pharmaceuticals. In fact, the rTG form that proves to be the best choice for clinicians is currently only available as a dietary supplement. Additionally, the United States Pharmacopeia (USP) has created a verification program which includes specifications for both fish oil raw materials and finished products. While products which are “USP Verified” are not technically “Pharmaceutical Grade,” these products are usually of the highest quality possible.

Recommendations for Selecting Therapeutic Marine Omega-3 Fatty Acid

- The best therapeutic option for delivering marine omega-3 fatty acids is a concentrated “bio-identical”- rTG form of fish oil. A single softgel containing rTG fish oil can easily provide well over 700 mg of EPA and DHA and rTG forms outperforms the EE version of fish oil in terms of bioavailability and raising omega-3 index.
- Prescription pharmaceutical omega-3 fatty acids are only available in an EE form. However, in the event that a person’s insurance is willing to pay for these products upon the diagnosis of severe hypertriglyceridemia (TG>500), these products have been shown to be safe and effective; albeit slightly less so than rTG forms available as dietary supplements. EE products should be consumed with a high-fat meal for best absorption, although it is important to instruct patients to add only healthy fats.
- Clinicians should consider having (or recommending) various blends of omega-3 products (some high in EPA, high in DHA, or a blend of EPA and DHA) to address different therapeutic targets. (see our paper addressing different therapeutic uses of EPA and DHA at www.pointinstitute.org)
- Clinicians can use blood testing (such as the omega-3 index) to determine the EPA and DHA status of patients and monitor dose accordingly.
- While Krill products appear to be adequate alternative sources of EPA and DHA, their low potency and high relative cost make them poor therapeutic substitutes for rTG fish oil, even if the suggested benefits of PL bioavailability can be proven in future clinical trials.
- Low dose fish oil products, while reliable sources of EPA and DHA are like krill oil in that they provide too low of a payload of EPA/DHA to be a viable therapeutic product. They are, however, a cost effective way to increase omega-3 fatty acids in low risk individuals desiring to augment an already healthy diet.
- Enteric-coated capsules increase the potential for ingesting unwanted chemical compounds and increasing the lot-to-lot variability in omega-3 absorption. Clinicians should ask their supplier for enteric-coating ingredients and lot specific dissolution information before relying on these products. The additional cost of enteric-coating is usually not warranted when using highly purified and concentrated oils.