A Comparison of Synthetic Ethyl Ester Form Fish Oil vs. Natural Triglyceride Form

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The benefits of EPA and DHA are well established

An abundance of evidence shows that increased intake of the long chain omega-3 fatty acids, EPA and DHA, can markedly reduce the risk of heart disease and other chronic diseases. EPA and DHA occur naturally in some fish species, as well as other marine organisms such as algae. However, most Americans eat very little fish and are deficient in long-chain omega-3 fatty acids[1]. In response, health care professionals are increasingly recommending purified fish oil as a dietary supplement.

These recommendations have driven the sales of fish oil supplements from $35 million in 1995 to approximately $310 million in 2005[2]. In 2006 alone, more than 200 new products enriched with omega-3 fatty acids were introduced to the market[3]. The majority of food products being introduced onto the market are actually enriched with plant-derived omega-3s, which are rich in alpha linolenic acid (ALA). Scientific evidence strongly suggests that these omega-3s from plant sources do not have the same beneficial effects as marine-derived EPA and DHA[4,5].

The U.S. has been slow to adopt a recommended daily allowance for EPA and DHA; however, nutrition experts from around the world have reached a consensus that adults benefit from consuming a minimum of 500 mg of combined EPA and DHA daily[6]. In addition, the American Heart Association recommends that individuals at defined risk for cardiovascular disease consume 1 g/day, and those with elevated triglycerides 2–4 g/day[7].

It is difficult for most individuals to maintain adequate levels of EPA and DHA intake from fish consumption alone. What's more, contamination of the ocean environment has made obtaining optimal levels of omega-3 fats by eating fish a potential health hazard. In fact, the FDA and the EPA have both warned the public of the potential dangers of consuming too much fish because of the associated toxins. Fish oil supplements may well be the healthier choice; studies have compared levels of mercury and organochlorines in fish versus purified fish oil supplements and concluded that the fish oil provides the benefits of EPA and DHA without the risk of toxicity[8,9].

For all of these reasons, fish oil supplements have become a vital part of nutritional and preventive medicine. And while there are hundreds of fish oil supplements available today, few consumers know that there are two distinctly different molecular forms of fish oil supplements on the market: one containing synthetic ethyl esters and the other containing natural triglycerides. More importantly, do these divergent delivery forms of EPA and DHA produce a meaningful difference in the availability of omega-3s to the body?

What are Triglycerides and Ethyl Esters?

The omega-3 fatty acids, EPA and DHA, occur naturally in fish in the triglyceride form. A triglyceride consists of a three-carbon [tri-] glycerol "backbone" with each carbon linked to a fatty acid. Thus, each triglyceride molecule contains three fatty acids. In non-concentrated fish oil, approximately 20–30% of the fatty acids are EPA and DHA. Conversely, a highly purified fish oil concentrate can contain from 60–85% EPA and DHA.

In making a fish oil concentrate, the individual fatty acids are first removed form the glycerol backbone by simple hydrolysis. After the fatty acids have been hydrolyzed from the glycerol backbone, they undergo molecular distillation, which allows for the relative concentrations of EPA, DHA, and other naturally occurring fatty acids to be modified. (Note: Free fatty acid forms of EPA and DHA have a very reactive free-electron in their structure and are highly unstable. For this reason, free fatty acid EPA and DHA are not used for human consumption because they are rapidly degraded via auto-oxidation.)

Once the desired amounts of fatty acids are achieved, a manufacturer chooses from two distinctly different options. The first is to use enzymes to reattach the fatty acids to the glycerol backbone in a process known as "re-esterification." This process reassembles the fatty acids into the natural triglyceride structure.

The second, less costly manufacturing option is to react the free fatty acids with ethanol (CH3CH2OH). This results in an "ethyl-ester" fatty acid, a fatty acid that does not occur naturally anywhere in the human diet[10]. The majority of concentrated EPA and DHA products available to consumers today are in the ethyl ester form, perhaps due to this lower cost of production.

The effects of ethyl-ester EPA and DHA formulas compared to triglyceride EPA and DHA formulas are only beginning to be investigated; thus, it is difficult to establish conclusively their relative bioavailability and activity in the body. Indeed, a search of the current published research on omega-3s does not reveal consensus in the scientific community on this issue. However, it has been established that several distinct differences exist with regard to how the body handles ethyl ester versus triglyceride EPA and DHA.

During normal fat digestion, pancreatic lipase acts on (hydrolyzes) a non-polar triglyceride to remove the fatty acids, resulting in two free fatty acids and a monoglyceride. After hydrolysis, the two fatty acids and monoglyceride become polar molecules (they carry an electric charge), which allows for them to be easily absorbed. For ethyl esters to be absorbed, the added ethyl molecule (CH2CH3) must be cleaved, presumably by pancreatic lipase, away from the original fatty acid. It has been shown that pancreatic lipase hydrolyzes fish oil ethyl esters 10 to 50 times more slowly than it does corresponding fish oil triglycerides[11]. The notion that ethyl ester fish oils are absorbed as well as triglycerides is in apparent contrast to the low rate of hydrolysis by pancreatic lipase.

In fact, preliminary studies have shown that ethyl ester fish oils are not absorbed in their manufactured synthetic form. Studies of both humans and animals have found that ethyl ester EPA and DHA do not appear in the blood of animals or humans, even after intake of a large amount (30 g) as a single dose in humans[12–14].

Clinical studies on bioavailability and other comparative issues

Six human trials have compared the absorption of ethyl ester (EE) and triglyceride (TG) fish oil supplements. Of the six studies, four concluded that the natural triglyceride form absorbs better, and the other two studies did not find a significant difference in absorption.

Lawson, *et al*[15] compared the relative absorption of TG, EE, and free fatty acids EPA and DHA in humans. Subjects were given 1 g EPA and 0.67 g DHA as a single dose. As free fatty acids, the EPA and DHA were absorbed at > 95%; as triglycerides, EPA was absorbed at 68% and DHA at 57%; as ethyl esters, EPA was absorbed 20% and DHA at 21%. When the authors repeated the study[16] with coingestion with a high fat meal (approx 40 g fat), the absorption of EPA (but not DHA) from the TG form improved from 68% to 90%. The ethyl ester form of both EPA and DHA improved from 20% to about 60%, which was still below absorption of the TG form before the addition of a high fat meal.

El Boustani, *et al*[17] investigated the kinetics of 1 g of EPA and the incorporation into plasma triglycerides after ingestion of four chemical forms: ethyl ester, free fatty acid, arginine salt, and triglyceride. When administered as ethyl ester, the EPA in plasma triglycerides was about three times less than in the other three forms and occurred three hours later. In all of the subjects studied, EPA incorporation was much greater after ingestion of the TG form than the EE form.

Beckermann, *et al*[18] also investigated the comparative bioavailability of EPA and DHA from triglycerides, ethyl esters, and free fatty acids in a randomized triple crossover design. EPA/DHA was administered in capsules as triglycerides (1.68/0.72 g), free fatty acids (1.35/1.065 g), and ethyl esters (1.86/1.27 g). The mean bioavailability of EPA/DHA was highest in the free fatty acid form while the mean bioavailability of ethyl ester EPA/DHA was only 40–48% compared to the triglyceride form. Maximal plasma levels were about 50% lower for ethyl esters when compared to triglycerides (free fatty acids were 50% higher than triglycerides.) In this study the tolerability of free fatty acids was much worse than that of triglycerides or ethyl esters because of fishy eructations (burps). This is consistent with the highly oxidizable nature of free fatty acids and their tendency to become rancid quickly.

In a related study, Visioli, *et al*[19] compared dietary fish, which contains only the natural triglyceride form EPA and DHA, to an equivalent dose of EPA and DHA as EE. Their data compared six weeks of eating 100 g/day of salmon, providing 383 mg EPA and 544 mg DHA, to capsules that contained 450 mg EPA and 318 mg DHA as ethyl esters. Plasma omega-3 concentration after salmon intake was found to be significantly higher when compared to ingestion of EE capsules.

Hong, *et al*[20] addressed a different question in their comparison study. Do these different omega-3 "molecular packages" have the same biological activity? The Hong group compared EE EPA and DHA to TG EPA and DHA on hepatic fatty acid oxidation in rats. Hepatic fatty acid oxidation is one of the mechanisms by which omega-3 fats improve lipid metabolism. The Hong study demonstrated that diets containing EPA and DHA ethyl esters do not mimic the physiological activity of a fish oil diet containing equivalent amounts of EPA and DHA as triglycerides in affecting hepatic acid fatty acid oxidation in rats. While not conclusive, this study suggests that the physiological activity of omega-3 fats may depend on their specific lipid structure.

Two early, small trials concluded that DHA and EPA from ethyl esters are equally well absorbed when compared to triglycerides. Nordoy, *et al*[21] compared the serum levels of EPA and DHA in five subjects after a single test meal of 27 g of EPA and DHA as 1) triglycerides, 2) ethyl esters, and 3) ethyl ester + 12 g olive oil. The subjects were given a 1–2 week wash out period between test meals to avoid carry over effect. No significant differences were observed in the serum EPA and DHA increases after the three different fish oil-containing meals. This study suggests that both ethyl esters of fish oil and triglycerides of fish oil were equally well absorbed when given in a liquid test meal containing 27 g of EPA and DHA.

The Nordoy team suggested that the differences between their findings and those of the Lawson and El Boustani groups is that higher amounts of fat and higher concentrations of EPA and DHA were used, which may have overcome the differences in the rate of pancreatic lipase activity on divergent molecular forms. Similar to the findings of Lawson, *et al*, the increase in plasma triglyceride concentration after intake of ethyl ester was highest when the meal was supplemented with 12 g of olive oil. The researchers concluded that the practical implications of their data is that there need be no concern about the absorbability of EPA and DHA from fish oil, either as ethyl ester or as triglyceride. However this may only apply to mega doses of EPA and DHA, similar to what was used in the trial.

Krokan, *et al*[22] administered similar amounts of EPA and DHA as ethyl esters and triglycerides in 40 healthy volunteers twice daily for 14 days. Volunteers were divided into five groups of eight volunteers, and received a daily dose of 1) 3.4 g ethyl esters, 2) 6.8 g ethyl esters, 3) 11.9 g ethyl esters, 4) 3.6 g triglycerides, 5) 7.3 g triglyceride. The investigators recorded

multiple serum values, including EPA and DHA in total serum lipids and in serum phospholipids, EPA/AA ratio, linoleic acid, saturated fatty acids, triglyceride levels, and cholesterol levels in each volunteer. The researchers also examined in vitro activity of porcine pancreatic lipase for the two different forms of omega-3 fats.

The rate of in vitro hydrolysis of the ethyl ester form of EPA and DHA was found to be significantly slower than the natural triglyceride form. However, Krokan, *et al* reported that in spite of differences in the rate of hydrolysis in vitro, equivalent doses of EPA/DHA in the form of ethyl ester or triglyceride give similar increases in plasma levels of EPA/DHA. It is noteworthy that the lead author of this study is also one of the inventors of US Patent #5502077 that pertains to the ethyl ester form of fish oil used in this trial (Ethyl Ester K85, Norsk Hydro)[23].

Stability of Ethyl Esters vs. Triglycerides

The therapeutic action and safety of fish oil is in part related to its molecular stability and resistance to oxidative damage. Fish oil that has been subject to oxidative damage may do more harm to the body than good[24]. EPA and DHA are long-chain polyunsaturated fatty acids (PUFAs), which means they contain several double bonds within their carbon-hydrogen chain. In each location of a double bond, there is vulnerability for free radical attack, which results in an oxidized and rancid oil. The potential negative health effects of consuming rancid fish oils have not been fully elucidated. However, it has been shown that oxidized by-products of polyunsaturated fatty acids, including DHA, are elevated in patients with neurodegenerative conditions[25].

The triglyceride structure is the natural "resting" state for lipid molecules. The inherent structure of three fatty acids attached to one glycerol backbone provides protection to the double bonds in the long-chain PUFAs from being exposed to free radicals. An ethyl ester fatty acid, on the other hand, exists as a single strand, and is exposed on all sides to free radicals. Although there is little data that directly compares the stability of EE fish oils to TG fish oils, such basic biochemistry suggests the superior stability of TG fish oils both inside a capsule or liquid as well as within the body. Resistance to oxidative damage is a critical quality of a fish oil supplement. Comparing the stability of EE and TG fish oil in vitro and in vivo is an important issue that warrants further investigation.

Conclusion

Although the majority of fish oil products on the market contain the ethyl ester form of EPA and DHA, current research evidence points toward the triglyceride form for better absorption and assimilation.

In addition, some proponents of EE fish oils declare that highly concentrated ethyl ester fish oils reduce the total intake of calories of fat that may be associated with consuming less concentrated natural triglyceride oils. This opinion does not take into account the studies that indicate that coingestion with a high fat meal is necessary to achieve reasonable absorption of ethyl ester form omega-3s.

The therapeutic value of EPA and DHA is based on several large-scale epidemiological studies that demonstrated that fish-consuming populations have a low incidence of cardiovascular disease[26–31]. All of the data from these large epidemiological studies are based on natural triglyceride fish oils. Conversely, several large-scale prevention studies, such as the GISSI-Prevenzione trial, have been successful using EE form EPA and DHA[32].

A global increase in the consumption of EPA and DHA has been proposed as a potential intervention to improve public health worldwide. In addition, health care practitioners are advising the use of EPA and DHA for a variety of conditions. Most consumers and health care professionals are unaware that there are two different delivery forms of these valuable nutrients.

It is fair to say that the scarcity of data concerning the relative bioavailability of ethyl ester and triglyceride forms of EPA and DHA does not allow for definitive conclusions, and more research is warranted. In addition, the issues of in vitro and in vivo stability of EE form fish oils as well as the possibility that the EE form cannot mimic the physiological activity of triglyceride form are of paramount importance to explore. Evaluating these issues before relying on a synthetic form of EPA and DHA merely seems prudent.

Ethyl Esters

- Do not occur naturally in the human diet
- Hydrolysis by pancreatic lipase 10–50 x slower
- May not mimic the physiologic activity of triglycerides
- Less expensive to produce
- Less molecularly stable

Triglycerides

- Natural form of dietary lipids
- Faster hydrolysis by pancreatic lipase
- Human digestion designed to absorb triglyceride form
- More expensive to produce
- More molecularly stable

The Physiology of Lipid Digestion

Approximately 98% of dietary lipids are triglycerides; the remainder consists of phospholipids, sterols, and fat-soluble vitamins. Because triglycerides are non-polar and insoluble in an aqueous environment, they require unique steps for proper absorption and assimilation.

The first step of lipid digestion occurs in the small intestine where fats are broken down physically, and held in suspension. Bile is secreted in the small intestine to break lipid aggregates into smaller particles, a process called emulsification. Within the lumen of the small intestine, a triglyceride molecule must be enzymatically digested by pancreatic lipase to yield a monoglyceride and two fatty acids. The resulting monoglyceride and fatty acids are polar (carry an ionic charge) and can efficiently diffuse into the enterocyte.

Enterocytes are cells that act as a barrier between the contents of the intestine and the blood stream. The major products of lipid digestion, fatty acids and 2-monoglycerides, enter the enterocyte by simple diffusion across the cell membrane. Once inside the enterocyte, fatty acids and 2-monoglycerides are transported into the endoplasmic reticulum, where they are used to resynthesize a triglyeride. The newly formed triglyceride is packaged with cholesterol, lipoproteins, and other lipids into particles called chylomicrons. This all occurs within the absorptive enterocyte of the small intestine.

Chylomicrons are transported first into the lymphatic vessel; then chylomicron-rich lymph drains through the lymphatic system, which rapidly flows into the blood.

Both TG and EE fatty acids must be reassembled into the TG form within the enterocyte for absorption. Some experts have postulated that the lack of glycerol backbone is the major obstacle to efficient absorption of EE form EPA and DHA. The EE form lacks the glycerol backbone needed to reassemble the TG structure. Therefore, the EE fatty acids that are hydrolyzed must find a glycerol backbone from another fatty acid donor. This would explain the noted improvement of EE absorption observed by Lawson, et al (see “Comparison studies”) when coingested with a high fat meal.

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