Complete Product Reference Guide
Table of Contents:

Supplement Facts Box

Introduction to Fish Oils

Health benefits of Omega-3 fatty acids

Quality of OrthoMega and other fish oils

Content of OrthoMega

Allergen information

What is Pharmaceutical Grade Fish Oil

Ethyl Esters versus Triglycerides

Commonly asked Questions
Supplemental Facts Box

**Supplement Facts**

<table>
<thead>
<tr>
<th>Serving Size: 1 Soft Gel</th>
<th>Servings Per Container: 120</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>1 Soft Gel Contains</th>
<th>Amount Per Serving</th>
<th>% Daily Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>420 mg</td>
<td>*</td>
</tr>
<tr>
<td>DHA</td>
<td>300 mg</td>
<td>*</td>
</tr>
</tbody>
</table>

* % Daily Value not established

Ingredients: Purified Marine Triglyceride Concentrate, Vitamin E Mixed Tocopherols, Gelatin, Glycerin and Purified Water.

Label

Orthomega™
CAPSULES
Molecularly Distilled, Bury-Free Omega-3 Fish Oil

Introduction

Omega-3 fatty acids have received a great deal of attention lately. The health benefits of Omega-3 fatty acids are just coming to light after years of research. The American Heart Association recognizes that consuming Omega-3 fatty acids derived from fatty fish benefit the hearts of healthy people and those at risk for cardiovascular disease. The AHA recommends that individuals eat fatty fish at least two times per week. While, for a number of different reasons, this is not always achieved, many people supplement their diets with fish oil products.

FDA allows the following health claim to be placed on fish oil products: “Supportive but not conclusive research shows that consumption of EPA and DHA omega-3 fatty acids may reduce the risk of coronary heart disease. One serving of [name of product] provides [x] grams of EPA and DHA omega-3 fatty acids”.
Health Benefits of Omega-3 fatty acids:

Omega-3 fatty acids have been shown to prevent primary and secondary cardiovascular events, reduce triglycerides, and help improve conditions associated with Metabolic Syndrome. They also exhibit anti-inflammatory effects and help those with depression and other mood disorders. Omega-3 fatty acids are also beneficial to developing children and can increase ocular and cognitive health. For more detailed information on the health benefits of Fish Oils, please see the attached JANA article.

Quality

There are a number of different fish oil products on the market today. Quality is a major concern for numerous consumers, labels and advertising that accompany many of these fish oil products are often times confusing and at times misleading. Ortho Molecular Products has set very high standards for our OrthoMega fish oil capsules (Specification Sheet Attached). OrthoMega has one of the highest EPA:DHA ratios available in capsule form, which makes patient compliance easier. OrthoMega exceeds the specifications set by the CRN (Council for Responsible Nutrition) monograph (Attached) in all areas of purity testing, including mercury. Ortho Molecular Products also has the only fish oil in the industry that is USP verified (Attached you will find a Frequently Asked Questions sheet about USP and The USP ingredient verification program process).

A quality fish oil product will have low p-Anisidine and peroxide values. Peroxide values are used to measure primary oxidation of fish oil and p-Anisidine values are used to measure secondary oxidation levels. High results indicate that the oil may be rancid. Ortho Molecular Products has set standards for p-Anisidine and peroxide levels that coincide with the CRN monograph and continuously exceed these specifications with each lot.

What kind of fish are in OrthoMega?

The fish used in OrthoMega include: Anchovy (Engraulis ringerns, 95-99%), Sardine (Sardinops sagax, 1-5%), and occasionally Mackerel (Scomber japonicus). The fish are from the cold deep waters off the coast of Peru. Fish taken from cold deep waters are more fatty and contain more Omega-3 fatty acids. The water where they are harvested has significantly less environmental impurities than in North America.

Allergen Information

Fish are recognized as allergens; however, the allergens in fish are proteins. A quality fish oil product will have very little protein matter left in the oil. The food Allergy Research And Resource Program Laboratory has tested the oil in OrthoMega for residual protein content. In their expert opinion fish oil containing less than 10 ppm of residual fish protein, presents no allergenic hazard to fish allergic consumers. OrthoMega contains less than 10 ppm of residual protein content.
What is Pharmaceutical Grade Fish Oil?

The term “pharmaceutical grade” fish oil was coined by Barry Sears in his book “The Omega Rx Zone”, and is not recognized by any standardization authorities, like USP. Many companies tout that their product is pharmaceutical grade, but this is merely a marketing scheme.

Ethyl Ester and Triglyceride forms of Fish Oil

Fish oil supplements are available in ethyl ester or triglyceride forms. Ethyl esters are formed by breaking the bonds of the original triglyceride molecule, concentrating the fatty acids, and then bonding the fatty acids to ethanol. Generally, ethyl ester fish oils allow for higher concentrations of Omega-3 fatty acids than triglycerides do. Triglyceride forms have the concentrated fatty acids esterified to a glycerol backbone to create a re-esterified triglyceride. Ortho Molecular uses the triglyceride form of Omega-3 fatty acids. There have been a small handful of studies done on the differences between ethyl esters and triglycerides. A few studies show that our bodies more readily absorb the triglyceride form, although this data is preliminary. For a complete review of ethyl esters versus triglyceride forms, please see the attached JANA article.

Commonly Asked Questions

Does OrthoMega contain any Vitamin E?
OrthoMega contains approximately 2.3 mg of mixed natural tocopherols per capsule. We do not label this in the supplemental facts box, but instead put it in the other ingredients section. The tocopherols are added to help preserve the fish oil and keep it from oxidizing.

What is the source of the gelatin used in OrthoMega?
The gelatin in OrthoMega is bovine.

Does OrthoMega contain any cholesterol?
OrthoMega contains approximately 0.28 mg of cholesterol per capsule.

What size bottle is OrthoMega available in?
OrthoMega comes in a 60, 120, and 180 count bottle. It is also available in a convenient 6 count blister package.
## ORTHOMEGA SPECIFICATION SHEET

<table>
<thead>
<tr>
<th>Product Name:</th>
<th>300/210 TG 1365 mg Fish Oil Capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product Code:</td>
<td>300210B1365</td>
</tr>
<tr>
<td>Vendor:</td>
<td>Ocean Nutrition Canada</td>
</tr>
<tr>
<td>Expiration Date:</td>
<td>1 year from date of receipt</td>
</tr>
</tbody>
</table>

### Specifications:

<table>
<thead>
<tr>
<th>Spec</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity analysis:</td>
<td>Spectral Identification</td>
</tr>
<tr>
<td>Free Fatty Acids:</td>
<td>Max. 1.5%</td>
</tr>
<tr>
<td>Acid Value:</td>
<td>Max. 3.0 mg of KOH/g</td>
</tr>
<tr>
<td>p-Anisidine Value:</td>
<td>Max. 20</td>
</tr>
<tr>
<td>Peroxide Value:</td>
<td>Max. 5 meq/Kg</td>
</tr>
<tr>
<td>Color:</td>
<td>Yellow</td>
</tr>
<tr>
<td>Moisture:</td>
<td>Max. 0.1%</td>
</tr>
<tr>
<td>Totox Number:</td>
<td>Max. 26</td>
</tr>
<tr>
<td>Average Fill Weight:</td>
<td>1129-1501 mg/capsule</td>
</tr>
<tr>
<td>Disintegration:</td>
<td>Max. 45 minutes</td>
</tr>
</tbody>
</table>

### Fatty Acid Profile:

<table>
<thead>
<tr>
<th>Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA mg/g (expressed as TG):</td>
<td>Min. 420 mg/capsule</td>
</tr>
<tr>
<td>DHA mg/g (expressed as TG):</td>
<td>Min. 300 mg/capsule</td>
</tr>
<tr>
<td>EPA mg/g (expressed as FFA):</td>
<td>Min. 400 mg/capsule</td>
</tr>
<tr>
<td>DHA mg/g (expressed as FFA):</td>
<td>Min. 290 mg/capsule</td>
</tr>
</tbody>
</table>

### Tocopherols:

<table>
<thead>
<tr>
<th>Tocopherol</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed Natural Tocopherols:</td>
<td>Min. 2.0 mg/g</td>
</tr>
</tbody>
</table>

### Microbiological Specifications:

<table>
<thead>
<tr>
<th>Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Count:</td>
<td>Max. 3000 CFU/g</td>
</tr>
<tr>
<td>Yeast/Mold:</td>
<td>Max. 300 CFU/g</td>
</tr>
<tr>
<td>Pseudomonas:</td>
<td>Negative</td>
</tr>
<tr>
<td>Salmonella:</td>
<td>Negative</td>
</tr>
<tr>
<td>S.aureus:</td>
<td>Negative</td>
</tr>
<tr>
<td>E.coli:</td>
<td>Negative</td>
</tr>
<tr>
<td>Coliforms:</td>
<td>Max. 10 MPN/g</td>
</tr>
</tbody>
</table>

### PCB and Heavy Metals:

<table>
<thead>
<tr>
<th>Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB's:</td>
<td>Max. 0.09 ppm</td>
</tr>
<tr>
<td>Dioxins:</td>
<td>Max. 2 pg WHC-PCDD/FTEQ/g</td>
</tr>
<tr>
<td>Arsenic:</td>
<td>&lt;0.1 ppm</td>
</tr>
<tr>
<td>Cadmium:</td>
<td>&lt;0.1 ppm</td>
</tr>
<tr>
<td>Lead:</td>
<td>&lt;0.1 ppm</td>
</tr>
<tr>
<td>Stontium:</td>
<td>Max. 0.5 ppm</td>
</tr>
<tr>
<td>Mercury:</td>
<td>Max. 0.01 ppm</td>
</tr>
</tbody>
</table>
### PRODUCT NAME:
Ortho Mega

### MANUFACTURED DATE:
12/18/2005

### RAW MATERIAL#:
M400

### RELEASE DATE:
2/8/2006

### LOT#:
20857

### EXPIRATION DATE:
12/08

### IDENTITY ANALYSIS
<table>
<thead>
<tr>
<th>METHOD</th>
<th>SPECIFICATION</th>
<th>RESULT</th>
<th>PASS/FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP 25 &lt;197&gt;</td>
<td>SOP# 4101</td>
<td>Spectral Identification</td>
<td>CONFORMS</td>
</tr>
</tbody>
</table>

### SPECIFICATIONS
<table>
<thead>
<tr>
<th>METHOD</th>
<th>MAXIMUM VALUE</th>
<th>RESULT</th>
<th>PASS/FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisidine Value</td>
<td>ISO/CD 6885.2</td>
<td>20</td>
<td>14.3</td>
</tr>
<tr>
<td>Peroxide Value</td>
<td>USP &lt;401&gt;</td>
<td>5 meq</td>
<td>0.10 meq</td>
</tr>
</tbody>
</table>

### EACH CAPSULE CONTAINS

<table>
<thead>
<tr>
<th>ACTIVE INGREDIENTS</th>
<th>CLAIM</th>
<th>%RDA</th>
<th>SPECIFICATION</th>
<th>RESULT</th>
<th>PASS/FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA Triglyceride</td>
<td>420mg</td>
<td>*</td>
<td>min 420mg</td>
<td>420mg</td>
<td>PASS</td>
</tr>
<tr>
<td>DHA Triglyceride</td>
<td>300mg</td>
<td>*</td>
<td>min 300mg</td>
<td>300mg</td>
<td>PASS</td>
</tr>
</tbody>
</table>

* % Daily Value Not Established

### MICROBIAL LIMITS TEST
<table>
<thead>
<tr>
<th>SPECIFICATION</th>
<th>RESULT</th>
<th>PASS/FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Plate Count</td>
<td>max 3000 CFU/g</td>
<td>&lt;10 CFU/g</td>
</tr>
<tr>
<td>Mold and Yeast</td>
<td>max 300 CFU/g</td>
<td>&lt;10 CFU/g</td>
</tr>
<tr>
<td>Coliforms</td>
<td>max 10 MPN/g</td>
<td>&lt;3 MPN/g</td>
</tr>
<tr>
<td>E. Coli</td>
<td>NEGATIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>NEGATIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Salmonella</td>
<td>NEGATIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>NEGATIVE</td>
<td>NEGATIVE</td>
</tr>
</tbody>
</table>

### TRACE HEAVY METALS

<table>
<thead>
<tr>
<th>MAXIMUM VALUE</th>
<th>RESULT</th>
<th>PASS/FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>&lt;0.1 ppm</td>
<td>COMPLIANT</td>
</tr>
<tr>
<td>Cadmium</td>
<td>&lt;0.1 ppm</td>
<td>COMPLIANT</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt;0.1 ppm</td>
<td>COMPLIANT</td>
</tr>
<tr>
<td>Mercury</td>
<td>&lt;0.01 ppm</td>
<td>COMPLIANT</td>
</tr>
</tbody>
</table>

### PESTICIDE RESIDUES

<table>
<thead>
<tr>
<th>MAXIMUM VALUE</th>
<th>RESULT</th>
<th>PASS/FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB's</td>
<td>0.09 ppm</td>
<td>COMPLIANT</td>
</tr>
<tr>
<td>Dioxins</td>
<td>2 ppt</td>
<td>COMPLIANT</td>
</tr>
</tbody>
</table>

### APPROVED BY/DATE:
Deborah Zurawski
Quality Manager
1. Is there a standard that identifies high quality long chain Omega-3 EPA and DHA products?

Yes. The Council for Responsible Nutrition (CRN) has sponsored a group of industry leaders to determine a consistent standard for measuring various quality indicators including oxidation, purity, and the amount of EPA and DHA in products, and to set standards for purity and stability of long chain Omega-3 EPA and DHA products. This standard is set forth in a voluntary monograph on long chain Omega-3 EPA and DHA.

2. What industry leaders participated in the working group?


3. Describe the monograph.

The voluntary monograph applies to long chain Omega-3 EPA and DHA obtained from fish, plant and microbial sources of marine algae. It sets forth rigorous and validated methodology for the assay methods measuring the amount of long chain Omega-3 EPA and DHA, as well as quality standards and limits on environmental contaminants.

4. Do all participants comply with the standards set forth in the monograph? How will compliance be recognized?

Compliance with the monograph is voluntary. Within the trade, products that comply with the monograph will be identified by comparing the certificate of analysis with the standards specified by the monograph. If companies choose to do so, they could highlight compliance with the monograph on the product label or product data sheets.
5. European monographs have already been developed for omega-3 products. How does this monograph differ?

This monograph specifically focuses on EPA and DHA and uses the *European Pharmacopoeia* assay methods to quantify these two fatty acids. The difference between the two monographs is that the European monograph includes other omega-3 fatty acids, in addition to EPA and DHA.

6. Why is cod liver oil excluded from the monograph?

An official monograph specifically for cod liver oil has previously been presented in the *United States Pharmacopeia*.

7. How are consistent measurements established? What is the process?

Six monographs were considered in establishing the quality standards set forth in this monograph for long chain Omega-3 EPA and DHA products. Based on a review of these available assay methods, the European Pharmacopoeia method to quantify EPA and DHA content in omega-3 products was used to provide a simple and uniform measurement of long chain Omega-3 EPA and DHA for product labeling. This method should guarantee at least 100% of stated content claim over the lifetime of the product.

8. What are the proposed standards for quality?

The quality of products is measured by the amount of free fatty acid (acid value), primary oxidation products (peroxide value), and secondary oxidation products (anisidine value) and total oxidation (TOTOX value).

Quality standards for long chain Omega-3 EPA and DHA products will measure: Acid Value, Peroxide Value (PV), Anisidine Value (AN) and TOTOX (a calculation based on (2 x PV) + AN).

The proposed limits for PV (5 meq/kg maximum) and anisidine value (20 maximum) represent the most stringent quality standards established for DHA and EPA products (see referenced monographs on next page).

The creation of a TOTOX value of 26 maximum further tightens quality standards while allowing manufacturers flexibility in individual PV and AN values.

9. What is TOTOX?

TOTOX is a measure of total oxidation calculated as a combined limit of peroxide and anisidine values.
10. Which environmental contaminants and heavy metals are measured by this monograph?

Environmental contaminants measured are dioxins (PCDDs and PCDFs) and PCBs. Heavy metals measured include Lead, Cadmium, Mercury and Arsenic. The limits set forth in the monograph are consistent with current or emerging European standards and with limits established under California’s Proposition 65.

11. What does this mean to me?

It means that when buying long chain Omega-3 products EPA and DHA, you can trust products that meet the standards set forth in this monograph to consistently report accurate amounts of long chain Omega-3 EPA and DHA, to be free of contaminants to a specified level, and to be stable for their labeled period of time.

---

VOLUNTARY MONOGRAPH

Council for Responsible Nutrition October 2002

Omega-3 DHA
Omega-3 EPA
Omega-3 DHA & EPA

DEFINITION

Omega-3 fatty acids, EPA and DHA, consist of the all cis forms of 5, 8, 11, 14, 17-eicosapentaenoic acid and 4, 7, 10, 13, 16, 19-docosahexaenoic acid, respectively. Omega-3 fatty acid products may be found with DHA as the predominant fatty acid, EPA as the predominant fatty acid or mixtures of DHA and EPA in varying combinations.

The content of omega-3 EPA and DHA is expressed as free fatty acid equivalents on a weight/weight basis, as mg EPA per gram or mg DHA per gram when available as single sources of omega-3. If a mixture of EPA and DHA exist in the product, the total omega-3 EPA and DHA content may be expressed individually and as total mg EPA and DHA per gram.

For other Omega-3 Fatty Acids see Assay method.

Food approved antioxidant may be added to enhance omega-3 EPA, omega-3 DHA, and omega-3 EPA and DHA product stability.

SCOPE

Applicable to omega-3 EPA and DHA fatty acids obtained from fish, plant, or microbial sources. Not applicable to cod liver oil. Omega-3 EPA and DHA may be found esterified as triglycerides, re-esterified as glycerides, or esterified as ethyl esters. Not applicable to free fatty acid product forms. Applicable to bulk oil product and encapsulated oil intended for use as dietary supplements. Not applicable to formulations, specialty delivery systems, and EPA and DHA concentrates >80% wt/wt. The specifications described herein apply throughout the stated lifetime (shelf-life) of the product.

CHARACTERISTICS

Long chain omega-3 EPA and DHA products are generally liquids at ambient temperature. The color varies from pale, light-yellow to orange. The products have a characteristic odor ranging from bland to mild fish-like.

IDENTIFICATION

Examine the chromatograms obtained in the assay for long chain omega-3 EPA and DHA. The presence of EPA and/or DHA based on retention time comparison to authentic reference standards establishes the identity of the product.
TESTS

Acid value. Maximum 3 mg KOH/g; AOCS Official Method Cd 3d-63

Peroxide value. Maximum 5 meq/kg; AOCS Official Method Cd 8-53

Anisidine value. Maximum 20; AOCS Official Method Cd 18-90

TOTOX. Maximum 26 (result of calculation, (2 x PV) + AV)

PCDDs and PCDFs. Maximum 2 pg WHO-PCDD/F-TEQ/g

Dioxin limits include the sum of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and are expressed in World Health Organization (WHO) toxic equivalents using WHO-toxic equivalent factors (TEFs). This means that analytical results relating to 17 individual dioxin congeners of toxicological concern are expressed in a single quantifiable unit: TCDD toxic equivalent concentration or TEQ.

PCBs. Total polychlorinated biphenyls (PCBs): Less than 0.09 mg/kg

Total PCBs should be expressed on a weight/weight basis and should include IUPAC congeners 28, 52, 101, 118, 138, 153 and 180, pending EU establishment of limits for four individual non-ortho PCBs and eight mono-ortho PCBs in 2004.

Sample preparation and appropriate methods of analysis for PCDDs, PCDFs, and PCBs are described in draft European Commission Directive “laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs”. Annex II of this document describes appropriate methods of analysis along with a specific list of PCDD, PCDF and PCB congeners to be included in the calculation. Gas chromatography coupled with high-resolution mass spectrometry has proved to provide required sensitivity and specificity. Identification of congeners should be performed according to EPA Method 1613 revision B: Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS or similar.

Heavy Metals.

Lead (Pb): Less than 0.1 mg/kg
Cadmium (Cd): Less than 0.1 mg/kg
Mercury (Hg): Less than 0.1 mg/kg
Arsenic (As): Less than 0.1 mg/kg

Note: The maximum intake of PCDDs, PCDFs, PCBs, other pesticides, and heavy metals in various parts of the world are based on Maximum Allowable Daily Levels (MADL) instead of absolute amounts in oil; therefore, appropriate consideration should be given to recommended daily serving and intake.
ASSAY

The assay used for quantitative determination of EPA and DHA content in omega-3 products is applicable to triglyceride and ethyl ester product forms with results expressed as mg DHA/g and mg EPA/g after correction to free fatty acid equivalents.

**EPA and DHA.** *Carry out the operations as rapidly as possible, avoiding exposure to actinic light, oxidizing agents, oxidation catalysts (for example, copper and iron) and air.*

Gas chromatography.

The assay is carried out on the methyl or ethyl esters of all-cis-eicosa-5,8,11,14,17-pentaenoic acid (EPA; 20:5 n-3) and all-cis-docosa-4,7,10,13,16,19-hexaenoic acid (DHA; 22:6 n-3) in the sample to be examined.

**Internal standard.** Methyl tricosanoate R.

**Test solution (a).** Prepare 3 solutions for each sample.

1. Dissolve the sample to be examined according to the table below and about 70.0 mg of the internal standard in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution.

<table>
<thead>
<tr>
<th>Approx. sum EPA + DHA</th>
<th>Amount sample to be weighed</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 – 50 %</td>
<td>0.4 – 0.5 g</td>
</tr>
<tr>
<td>50 – 70 %</td>
<td>0.3 g</td>
</tr>
<tr>
<td>70 – 80 %</td>
<td>0.25 g</td>
</tr>
</tbody>
</table>

Ethyl esters are now ready for analysis. For triglycerides continue as described in 2.

2. Introduce 2.0 ml of the solution obtained from step 1 into a quartz tube and evaporate the solvent at 40-50°C with a gentle current of nitrogen R. Add 1.5 ml of a 20 g/l solution of sodium hydroxide R in methanol R, cover with nitrogen R, cap tightly with a polytetrafluoroethylene-lined cap, mix and heat on a steaming water-bath for 7 min. Allow to cool to 40-50°C.

3. Add 2 ml of boron trichloride-methanol solution R, cover with nitrogen R, cap tightly, mix and heat on a water-bath for 30 min. Cool to 40-50°C, add 1 ml of trimethylpentane R, cap and shake vigorously for at least 30 s. Immediately add 5 ml of a saturated solution of sodium chloride R, cover with nitrogen R, cap and shake vigorously for at least 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer once more with 1 ml of trimethylpentane R. Wash the combined trimethylpentane extracts with 2 quantities, each of 1 ml, of water R and dry over anhydrous sodium sulphate R.
Test solution (b). (to be prepared at the same time as test solution (a))

Dissolve 0.300 g of the sample to be examined in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution. Proceed as described for test solution (a).

Reference solution (a). Prepare 3 individual solutions (to be prepared at the same time as test solution (a))

Dissolve 60.0 mg of docosahexaenoic acid ethyl ester CRS, about 70.0 mg of the internal standard and 90.0 mg of eicosapentaenoic acid ethyl ester CRS in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution. For analysis of ethyl esters the solutions are now ready for analysis. For analysis of triglycerides continue as described in step 2 for preparation of test solution (a).

Reference solution (b). (for system suitability of recovery vs. the theoretical response of the Flame Ionisation Detector (FID))

Introduce 0.3 g of methyl palmitate R, 0.3 g of methyl stearate R, 0.3 g of methyl arachidate R and 0.3 g of methyl behenate R into a 10 ml volumetric flask, dissolve in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution.

Reference solution (c). (for system suitability of chromatographic resolution)

Introduce a sample containing about 55.0 mg docosahexaenoic acid methyl ester CRS and about 5.0 mg of 15-tetracosenoic acid methyl ester CRS diluted to 10.0 ml of a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R.

Column:
— material: fused silica
— dimensions: l = at least 25 m, Ø = 0.25 mm,
— stationary phase: bonded polyethylene glycol polymer (film thickness 0.2 µm).

Carrier gas: hydrogen for chromatography R or helium for chromatography.

Split: 1:200, alternatively splitless with temperature control (samples need to be diluted 1:200 with a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R before injection)

1 CP-Wax 52CB, 25 m x 0.25 mm I.D. 0.2. µm film thickness, Chrompack cat. no. 7713 or equivalent will be suitable
### Temperature:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td></td>
</tr>
<tr>
<td>0 – 2</td>
<td>170</td>
</tr>
<tr>
<td>2 – 25.7</td>
<td>170 - 240</td>
</tr>
<tr>
<td>25.7 - 28</td>
<td>240</td>
</tr>
<tr>
<td>Injection port</td>
<td>250</td>
</tr>
<tr>
<td>Detector</td>
<td>270</td>
</tr>
</tbody>
</table>

**Detection:** flame ionisation.

**Injection:** twice 1 µl of each solution.

The assay is not valid unless:

- the chromatogram obtained with reference solution (b) gives area per cent compositions increasing in the following order: *methyl palmitate, methyl stearate, methyl arachidate, methyl behenate*; the difference between the percentage area of *methyl palmitate* and that of *methyl behenate* is less than 2 area per cent units,

- the chromatogram obtained with reference solution (c) shows 2 resolved peaks corresponding to *docosahexaenoic acid methyl ester CRS* and *15-tetracosenoic acid methyl ester CRS*, giving a chromatographic resolution of minimum 1.2,

- the chromatogram obtained with test solution (a) shows a resolution of *methyl tricosanoate R* and any *heneicosenoic acid methyl* ester present when compared with the chromatogram obtained with test solution (b) (if not, a correction term has to be used),

- experiments using the method of standard additions to test solution (a) show more than 95 per cent recovery of the added *eicosapentaenoic acid ethyl ester CRS* and *docosahexaenoic acid ethyl ester CRS*, when due consideration has been given to the correction by the internal standard.
Calculate the content of EPA and DHA as mg fatty acid/g oil using the following expression and taking into account the certified value of the reference substances. The results should be rounded to the nearest 10 mg/g based on the method’s precision.

\[
A_x \leftrightarrow \frac{A_x}{A_1} \leftrightarrow \frac{m_1}{C} \leftrightarrow \frac{1}{A_{x,r}} \leftrightarrow \frac{m_2}{m_3} \leftrightarrow 1000
\]

\[m_1 = \text{mass of the internal standard in test solution (a), in milligrams,}\]
\[m_2 = \text{mass of the sample in test solution (a), in milligrams,}\]
\[m_3 = \text{mass of the internal standard in reference solution (a), in milligrams,}\]
\[m_{x,r} = \text{mass of eicosapentaenoic acid ethyl ester CRS or docosahexaenoic acid ethyl ester CRS in reference solution (a), in milligrams,}\]
\[A_x = \text{area of the peak corresponding to eicosapentaenoic acid ester or docosahexaenoic acid ester in the chromatogram obtained with test solution (a),}\]
\[A_{x,r} = \text{area of the peak corresponding to eicosapentaenoic acid ester or docosahexaenoic acid ester in the chromatogram obtained with reference solution (a),}\]
\[A_1 = \text{area of the peak corresponding to the internal standard in the chromatogram obtained with test solution (a),}\]
\[A_3 = \text{area of the peak corresponding to the internal standard in the chromatogram obtained with reference solution (a),}\]
\[C = \text{a conversion factor to fatty acids based on the difference in molecular weight of ethyl esters in the standard and fatty acid}\]

\[C_{EPA} = 0.915\]
\[C_{DHA} = 0.921\]
**Total omega-3-acids.** From the assay for EPA and DHA, calculate the content of the total omega-3-acids using the following expression and identifying the peaks from the chromatograms:

\[
EPA + DHA + \frac{A_{n-3}}{A_{EPA} + A_{DHA}} \cdot (EPA + DHA)
\]

- \(EPA\) = content of EPA obtained from the assay for EPA and DHA,
- \(DHA\) = content of DHA obtained from the assay for EPA and DHA,
- \(A_{n-3}\) = sum of the areas of the peaks corresponding to C18:3 n-3, C18:4 n-3, C20:4 n-3, C21:5 n-3 and C22:5 n-3 methyl esters in the chromatogram obtained with test solution (b),
- \(A_{EPA}\) = area of the peak corresponding to EPA methyl ester in the chromatogram obtained with test solution (b),
- \(A_{DHA}\) = area of the peak corresponding to DHA methyl ester in the chromatogram obtained with test solution (b).
USP Fact Sheet

What is the United States Pharmacopeia?

The United States Pharmacopeia (USP) is a nonprofit, nongovernmental, standards-setting organization that advances the public health by ensuring the quality and consistency of medicines, promoting the safe and proper use of medications, and verifying ingredients in dietary supplements. Standards are developed by a unique process of public involvement and are accepted worldwide. USP achieves its goals through the contributions of volunteers representing pharmacy, medicine, and other health care professions, as well as science, academia, the U.S. government, the pharmaceutical industry, and consumer organizations. USP’s Internet address is www.usp.org.

What does USP do?

USP’s activities and initiatives revolve around several focus areas:

Publications and Standards. Establishing standards is a core USP activity. Currently, USP provides standards for more than 4,000 prescription and non-prescription drugs, dietary supplements, veterinary drugs, and health care products. These standards are published in the United States Pharmacopeia and National Formulary (USP–NF), which are officially recognized in the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 321 et seq.). USP also produces over 1,700 Reference Standards, which are an integral part of USP’s standards program. In addition, USP offers a Pharmacopeial Education program to provide continuing education courses for professionals working in the pharmaceutical industry – helping those who use the USP–NF better understand pharmacopeial processes, standards, tests, and methods.

Patient Safety. USP operates two medication error reporting programs: the Medication Errors Reporting (MER) Program and MEDMARXSM. The MER program is operated in collaboration with the Institute for Safe Medication Practices. MEDMARX is an Internet-accessible program for collecting, analyzing and reporting medication errors and adverse drug reactions anonymously. MEDMARX is used by subscribing hospitals and health systems as part of their quality improvement activities.

Verification Program. The Verification Program for dietary supplements was developed in response to the increasing concerns expressed about dietary supplements in the marketplace. Through compliance testing and document review, adherence to good manufacturing practices (GMPs), and post-marketing surveillance, the USP Verification Program is designed to help ensure that dietary supplement products contain the ingredients and quantities listed on the label. USP also operates an Ingredient Verification program that verifies ingredients used to manufacture and market dietary supplements in the United States and around the world.

Health Care Information. USP is actively working with its volunteer Expert Committees to provide health care information to improve the use of medicines. Recently, USP was called upon in the Medicare Modernization Act to create Model Guidelines (a list of drug categories and classes) for the Medicare prescription drug benefit. Having successfully completed this initial activity, USP is building an ongoing relationship with the Centers for Medicare and Medicaid Services (CMS) to revise the Model Guidelines to reflect new drug approvals, new therapeutic uses of existing drugs, and other new information. In addition to the Model Guidelines activity, USP will also be developing peer-reviewed articles on drug information for publication in the Annals of Internal Medicine.

Global Assistance Initiatives (GAI). USP’s GAI program consists of numerous global initiatives. USP was awarded several grants by the United States Agency for International Development's (USAID) Bureau for Global Health (BGH). Currently, USAID is supporting the USP Drug Quality and Information (USP DQI) program, which has programs in Ecuador, Peru, Paraguay, Bolivia, Mekong Delta region, Nepal, Romania, Russia, Mozambique, Madagascar, Ghana and Senegal.

Who are USP’s members and volunteers?

About 650 elected scientists and practitioners comprise USP’s scientific decision-making body by serving as members of the Council of Experts (CoE) or on expert committees. An additional 11 elected officers and trustees have fiduciary responsibilities for the management and policies of the organization. About 400 members represent state associations and colleges of medicine and pharmacy; the federal government; national and international professional, scientific, and trade organizations; the pharmaceutical industry; and consumer organizations. USP members meet once every five years to adopt resolutions and elect the CoE and Board of Trustees. The next member meeting will be held in 2010.
The USP Ingredient Verification Program

Overview
The United States Pharmacopeia’s Ingredient Verification Program (USP-IVP) verifies ingredients used to manufacture and market dietary supplements in the United States and around the world. It is a natural progression of USP’s long history of establishing officially recognized public standards for medicines and dietary supplements. USP has been a trusted and recognized source of standards for identity, strength, quality and purity of medicines and dietary supplements since 1820.

The program verifies ingredients such as vitamins, minerals, amino acids, botanical extracts and nonbotanicals as well as other ingredients used as either an active or inactive ingredient in the manufacture of dietary supplement products.

The USP-IVP process includes:
• Evaluation of a manufacturer’s quality systems through an audit for compliance with Good Manufacturing Practices (GMPs);
• Review of manufacturing and quality control documents for ingredients submitted for verification;
• Laboratory evaluation of ingredient samples from USP selected lots for compliance with label claim and program requirements;
• Granting of the USP-IVP verification mark; and
• Post-verification surveillance testing of ingredients bearing the USP-IV mark.

The USP-IVP Verification Mark
The use of the USP-IVP verification mark is granted for ingredients that meet the program’s requirements for verification. The mark indicates the verification of an ingredient’s quality by a trusted and established authority, which is USP. The mark provides dietary supplement manufacturers assurance that:
• The manufacturer’s quality system helps to ensure that the ingredient evaluated meets its label or certificate of analysis claim for identity, strength, purity and quality and that it is consistent in quality from batch-to-batch;
• The ingredient is prepared under accepted manufacturing practices; and
• The ingredient meets requirements for acceptable limits of contamination.

Participation
Participation in the program is voluntary and open to companies manufacturing fine chemicals and/or ingredients marketed for use in the dietary supplement industry in the United States or abroad.
Frequently Asked Questions

What are EPA and DHA?

- Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are biologically active Omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs).
- They are key building blocks in your cells and are needed throughout your whole body for good health.

How is Omega-3 fish oil different from plant-derived Omega-3?

- Plant-derived Omega-3 PUFA is in the form of alpha-linoleic acid (ALA).
  - ALA is considered an essential fatty acid (EFA) because the human body cannot synthesize it. The body converts ALA into EPA and DHA, which are biologically important to health.
  - Omega-3 PUFAs EPA and DHA are derived from ALA through enzymatic reactions in the body but this process does not efficiently supply the body with adequate amounts of EPA and DHA.
- EPA and DHA are found in fatty fish including Mackerel, Herring, and Salmon, and commercially available Omega-3 oils derived from these fish.
  - The ALA to EPA/DHA conversion process is very inefficient, therefore consumption of fish or Omega-3 fish oil is necessary to ensure the body receives enough EPA and DHA to grow and function properly.
What are the health benefits of Omega-3?
Omega-3s EPA and DHA are important building blocks in every cell in your body. As you grow and develop you need these as essential components of your diet. Omega-3s EPA and DHA are:

- An integral part of our cell membranes.
- Generally recognized for their cardiovascular, anti-inflammatory, brain, nervous system, and eye health benefits.
- Precursors of several important cellular messengers.

What is the recommended daily intake?
In general, 500 mg to 650 mg of Omega-3 EPA/DHA per day is recommended. (ISSFAL)

What is the source of MEG-3™ fish oil?
- MEG-3™ fish oil is sourced from the pristine wild Sardine, Anchovy, and Mackerel fishes in cold, pristine, deep waters off South America, where there are significantly less environmental impurities.
- MEG-3™ fish oil is the product of a healthy, fully sustainable fishery and is a renewable resource.

Does MEG-3™ set the industry standard for quality?
- Yes, MEG-3™ is the most trusted source of EPA/DHA fish oil. MEG-3™ is the first fish oil ingredient to achieve US Pharmacopeia (USP) verification, which is the most rigorous quality assurance verification in the world.
- MEG-3™ fish oils are manufactured to GMP (Good Manufacturing Practices) and HACCP (Hazard Analysis and Critical Control Point) standards and are also in compliance with the Canadian Food Inspection Agency (CFIA) and the Council for Responsible Nutrition (CRN) standards.

What does the term “Pharmaceutical-grade” fish oil mean?
- The term “Pharmaceutical-grade” fish oil is a commonly used marketing term to describe fish oil ingredients and is not recognized by standardization authorities, such as USP.
- MEG-3™ is the first fish oil ingredient to achieve USP verification, thus setting the standard for quality.

How is MEG-3™ concentrated fish oil made?

What is the difference between ethyl ester (EE) and triglyceride (TG) forms of Omega-3 concentrates?
MEG-3™ concentrated fish oil ingredients are available in either EE or TG form.
- Ethyl esters result from removing the glycerol backbone through a process called ethylation.
- The TG form consists of either unconcentrated Omega-3 fish oil (18:1/12) or concentrated Omega-3 fish oil that has been reconstituted or re-esterified to the TG form.
Most scientific literature shows our bodies have comparable absorption of omega-3 from EE or TG.

What is molecular distillation?
Molecular distillation is a refinement process for the concentration and purification of EPA and DHA from sardine fish oil material. As in any distillation process, it is based on molecular weight fractionation.

What is the significance of peroxide values (PV) and p-anisidine values (P-AV)?
Peroxide values (PV) are used to measure primary oxidation of the fish oil and p-anisidine values (P-AV) are used to measure secondary oxidation. Increased PV and P-AV numbers reflect increased oxidation of the oil. Ocean Nutrition Canada measures both values to have a complete picture of MEG-3™s oxidation status, as part of our rigorous quality assurance processes.
What is the difference between area percent and mg/g?

Omega-3 fish oil ingredients can be characterized either by area percent or mg/g of EPA and DHA.

- Area percent EPA and DHA is a comparison of EPA and DHA to all other fatty acids that may be in the fish oil. It is based on a 24-peak analysis on a gas chromatograph (GC) and does not directly correlate with mg/g values.

- The mg/g measure is based on a weight percentage of EPA and DHA compared to that of the fish oil, which takes into account the materials that would not show up on the GC (e.g. glycerin).

For example, the chart below outlines the area percent and mg/g for a number of MEG-3™ fish oil ingredients.

<table>
<thead>
<tr>
<th>MEG-3™</th>
<th>Area % EPA</th>
<th>Area % DHA</th>
<th>Minimum EPA (mg/g)</th>
<th>Minimum DHA (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40/20EE</td>
<td>40</td>
<td>20</td>
<td>340</td>
<td>170</td>
</tr>
<tr>
<td>40/20TG*</td>
<td>40</td>
<td>20</td>
<td>320</td>
<td>160</td>
</tr>
<tr>
<td>03/55EE</td>
<td>03</td>
<td>55</td>
<td>25</td>
<td>490</td>
</tr>
</tbody>
</table>

What are mixed natural tocopherols?

Mixed natural tocopherols (MNT), including d-alpha tocopherol (Vitamin E), are antioxidants, which prevent the oxidation and degradation of the oil, thereby extending the shelf life of the product.

How relevant is cholesterol content in MEG-3™ fish oil ingredients?

A daily dose of MEG-3™ concentrated fish oil contains less than 1% of the maximum recommended daily level of cholesterol. For example, MEG-3™ 40/20EE averages 0.21mg/g cholesterol, which is 0.07 % of the maximum daily recommended cholesterol intake of 300mg.
The Use of Fish Oil Supplements in Clinical Practice: A Review

Thomas G. Guilliams, PhD
Director of Science and Regulatory Affairs - Ortho Molecular Products, Inc.,
Stevens Point, Wisconsin
Clinical Instructor - University of Wisconsin-Madison School of Pharmacy

Duplication in whole or part is not permitted without permission.
The Use of Fish Oil Supplements in Clinical Practice: A Review

Thomas G. Guilliams, PhD*
Director of Science and Regulatory Affairs- Ortho Molecular Products, Inc.,
Stevens Point, Wisconsin
Clinical Instructor- University of Wisconsin-Madison School of Pharmacy

ABSTRACT

Increasing dietary consumption of fish high in omega-3 (n-3) fatty acids is well established as a way to improve numerous health outcomes. The prevention of both primary and secondary cardiovascular events, as well as intervention for such unrelated outcomes as depression and rheumatoid arthritis are now linked with n-3 fatty acid intake. Increasing fish consumption is neither an exact science, nor without risk of consuming toxins of various kinds. The advent of highly purified fish oil supplements, now widely available, has allowed very high levels of n-3 fatty acid consumption for both preventative and therapeutic clinical use. This review will focus on the data concerning fish consumption, fish oil supplements and their fatty acids as it pertains to clinical outcomes, with an emphasis on cardiovascular health.

BACKGROUND

In the early 1970s, it was observed that high levels of fat intake in the form of long-chain omega-3 fatty acids in Greenland Eskimo populations resulted in fewer cardiovascular events than Western populations who ingested less total dietary fat. In fact, these studies and others prompted the scrutiny of fatty acids based upon whether they were omega-3 (n-3), omega-6 (n-6) or omega-9 (n-9). Fatty acids in the n-3 and n-6 families are considered essential to humans because our metabolism is unable to de-saturate (make a double-bond) between carbons-3 and 4 (n-3) or between carbons 6 and 7 (n-6); counting from the omega or last carbon (See Figure 1 for basic fatty acid information). Typical Western diets provide much in the way of polyunsaturated fatty acids from vegetable sources, which supply high levels of n-6 fatty acids. Data from numerous epidemiological studies have suggested that lowering one's ratio of n-6/n-3 in the range of 3:1 to 6:1 (typical American diet may be as high as 20:1) will have great health benefits. The creation of trans-fatty acids through food processing and cooking further complicates the issues both metabolically and epidemiologically.

N-3 FATTY ACIDS

Alpha-linolenic acid (ALA) is an n-3 essential fatty acid found primarily in certain seeds and green leafy vegetables. Flaxseeds are one of the richest sources of ALA. Converting this 18 carbon fatty acid to the 20 and 22 carbon fatty acids found primarily in fish oils requires several steps of elongation and de-saturation (see Fig. 1), reported to be a very inefficient process in adults, suggesting that direct consumption is more reliable. And while some data suggests that ALA may help prevent secondary cardiovascular events, most of the focus on n-3 fatty acid research is with consumption of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) from fish and fish oil supplements. In humans, the retroconversion between ingested DHA to plasma EPA seems to be higher than the conversion of EPA to DHA.
Figure 1:

**OMEGA-3 FATTY ACIDS**

**STRUCTURES**

- Alpha-Linolenic Acid (ALA) 18:3 n-3
- Eicosapentanoic Acid (EPA) 20:5 n-3
- Docosahexaenoic Acid (DHA) 22:6 n-3

**NAMING FATTY ACIDS**

- Common Name
- Shorthand
  - Oleic
  - Linoleic
  - 18:1 n-9
  - 18:2 n-6

**Typical Fatty Acid Profile of Various Oils & Cooked Fish**

<table>
<thead>
<tr>
<th></th>
<th>Saturated</th>
<th>Mono-unsaturated</th>
<th>Polyunsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Butter</td>
<td>42%</td>
<td>26%</td>
<td>12%</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>11%</td>
<td>72%</td>
<td>25%</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>10.9%</td>
<td>1.8%</td>
<td>24.2%</td>
</tr>
<tr>
<td>Canola Oil</td>
<td>4%</td>
<td>1.8%</td>
<td>56.1%</td>
</tr>
<tr>
<td>Coconut Oil</td>
<td>58.7%</td>
<td>8.2%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Flax Seed Oil</td>
<td>4.5%</td>
<td>18%</td>
<td>15%</td>
</tr>
</tbody>
</table>

**EPA Ethyl Ester**

**DHA**

**EPA**

**Triglyceride**

**n-3 and n-6 FATTY ACIDS COMPETE FOR ENZYMES DURING METABOLISM**

- 18:3 n-3 ALA
- 18:4 n-3 SDA
- 20:4 n-3 ETA
- 20:5 n-3 EPA
- 22:6 n-3 DHA

**ESSENTIAL FATTY ACIDS**

- Delta 6 desaturase
- Elongase
- Delta 5 desaturase

**SDA - Stearidonic Acid, ETA - Eicosatetraenoic acid, DGLA - Dihomogammalinolenic Acid, COX - Cyclooxygenase**

*Data from USDA Nutrient Data Laboratory website (http://www.nal.usda.gov/fnic/foodcomp). Accessed January 8th, 2005*
CARDIOVASCULAR USES

Primary Cardiovascular Event Prevention

Numerous reviews have summarized the cardiovascular benefits of fish and fish oil consumption.6,7,8,9,10 The data concerning primary prevention, however, is less straightforward than the data relating to secondary prevention. In several large cohort studies, the relative risk for CHD and sudden death is reduced with increased fish consumption in men and women.11,12,13,22,27 While others showed no statistical differences based on fish consumption.14,15 Plasma EPA and DHA levels measured upon initiation of the Physicians’ Health Study did not relate inversely with incidence of myocardial infarction,16 however in this same group both fish consumption, based on dietary questionnaire, and blood n-3 levels were statistically related to reduced risk of sudden cardiac death.17,18 In this cohort of 20,551 men, the multivariate relative risk for sudden cardiac death in those consuming 1 fish meal per week was 0.48, compared with men who consumed fish less than once per month.17 The adjusted relative risk in the 4th quartile of red cell n-3 levels was 0.19.18

The Honolulu Heart Program, following Japanese-Americans living in Hawaii, found that the relative risk for CHD mortality was cut in half for heavy smokers (>30 cigs/day) if they consumed greater than 2 fish meals per week.19 Siscovick20 reported that in a population-based case-control study in King County, WA that both dietary intake of seafood containing n-3 fatty acids and red blood cell membrane n-3 fatty acid concentrations were inversely related to primary cardiac arrest. Both of these associations were dose-related. Among the Nurses’ Health Study cohort, fatty fish intake was associated with a reduced risk of thrombotic stroke, while there was no increased risk for hemorrhagic strokes in women.183 A similar large cohort in the Physicians’ Health Follow-up study found the same lowered risk of stroke with fish consumption in men.184 The American Heart Association recommends that patients without documented coronary heart disease (CHD) eat a variety of (preferably fatty) fish at least twice a week, including oils and foods rich in alpha-linolenic acid (flaxseed, canola and soybean oils; flaxseed and walnuts).9,21

Secondary Cardiovascular Event Prevention

One of the first studies to assess the secondary prevention potential of n-3 fatty acids from fish was the diet and reinfarction trial (DART).23 The men randomized to receive advice to increase fatty fish consumption (others were advised to increase fiber or reduce fat intake) after recovery from MI, had a 29% reduction in 2-year all cause mortality. Unfortunately, like many lifestyle changes, this advice was difficult to maintain over many decades and both compliance and benefits seem to have been diminished after a decade.24

The largest secondary prevention trial to date is the GISSI-prevention trial.25 In this study, over 11,000 patients (surviving a recent MI) were randomized to receive 1 g/day n-3 fatty acids (capsules containing a minimum of 850 mg EPA and DHA as ethyl esters), 300 mg of vitamin E (acetyl d,l-alpha tocopherol), both or placebo. Most of these patients were concomitantly on cardiovascular pharmaceuticals of various kinds, as well as advised about diet and lifestyle changes. Total (RR=0.59) and cardiovascular mortality (RR=0.66) were significantly reduced in the fish oil group as early as 3 and 4 months into the study, respectively. The most dramatic reduction was in sudden deaths, for which relative risks of 0.37 (after 9 months) and 0.55 (42 months) were reported.26 Among the lipids measured, only triglyceride levels showed significant improvements. In all, there are over 20 randomized, placebo-controlled trials of dietary n-3 fatty acid from fish in CHD patients. A meta-analysis28 of these trials shows a 3-year average reduction of all cause mortality of 16% and death from MI of 24%. The American Heart Association recommends that patients with documented CHD consume about 1 g of EPA+DHA per day, preferably from fatty fish; EPA+DHA supplements could be considered in consultation with a physician.9,21

Anti-arrhythmic Effects of Fish Oils

Both primary and secondary prevention studies showed that n-3 fatty acid intake was profoundly better at preventing sudden deaths than reducing the incidence of non-fatal MI. Over 50% of the deaths attributed to CHD are sudden deaths (within 1 hour) caused by sustained ventricular arrhythmias. These data suggested that n-3 fatty acids may have anti-arrhythmic effects which initially do not lower the incidence of MI, but prevent many of these events from becoming fatal.10 This anti-arrhythmic effect has been reported in several animal and cell culture models.29,30 It is fairly well established that the incorporation of EPA and especially DHA within the plasma membrane of electrically excitable cardiac tissue changes membrane fluidity and modulates the actions of ion channels to prevent the destabilization that permits arrhythmias and ventricular tachycardia.31,34

One small pilot study was conducted with 10 patients who had implanted cardioverter defibrillators and repeated episodes of documented, sustained ventricular tachycardia.38 Compared with baseline, after these patients were infused with n-3 fatty acids, sustained ventricular tachycardia was non-inducible in 5 of the 7 (3 of the 10 patients who ate significantly more dietary fish were non-inducible at baseline). More research, including controlled trials, needs to be done using oral doses of fish oil preparations and clinical outcomes. Leaf et al.10 recommend that those with a family or personal history of CHD should supplement their diets with 600 mg of EPA plus DHA, and higher, 1 to 2 grams, if there is also a family history of sudden cardiac death.

Reducing Triglycerides

Elevated triglycerides (TG), both fasting and postprandial, are directly related to the progression of atherosclerosis and are considered independent risk factors for CHD,
especially in women. Long-chain n-3 fatty acids from fish like EPA and DHA have shown consistent TG lowering effects in both animals and humans. A meta-analysis of 65 published reports showed TG reduction averaging 25% was typical with fish oil consumption (mean dose 4 g/day EPA + DHA) in both normolipidemic and hypertriglyceridemic subjects. These data also show a dose-response relationship between fish oil intake and triglyceride lowering as well as a slight rise in LDL cholesterol (5-10%) and a smaller elevation in HDL cholesterol (1-3%).

Post-prandial (after a fatty meal) plasma TG levels may even be more correlated to atherosclerotic progression than fasting TG levels. Chronic intake of n-3 fatty acids from fish has been shown to reduce post-prandial plasma TG levels. A recent study showed that exercise when combined with fish oils was additive in post-prandial TG lowering. Ten healthy recreationally-active subjects in a cross-over design were tested for changes in fasting and post-prandial (after 1,000 calorie shake- 99% fat after 12-hour fast) TG levels after 5 weeks of fish oil supplementation (4 g/day in 8 capsules of 300 mg EPA and 200 mg DHA each) or exercise (60% VO2max on treadmill for 1 hour), both or neither (control). When exercise was added to fish oil supplementation, the peak plasma TG levels went from 38% reduction (fish oil vs. control) to 50% reduction (fish oil + exercise vs. control). Total area under the TG curve was reduced from 27% to 42% respectively. While both EPA and DHA seem to have triglyceride lowering benefits, DHA may have a more favorable effect. The American Heart Association recommends that under a physician’s care, patients who need to have triglyceride lowering benefits, DHA may have a more favorable effect. The American Heart Association recommends that under a physician’s care, patients who need to lower triglycerides should consume 2 to 4 grams of EPA+DHA per day provided as capsules.

Other Cardiovascular Risk Factors

In general, fish oil supplements have a favorable, but small effect on HDL cholesterol levels (1-5%). Combined with the more widely observed TG lowering, this (this what?) improves the important TG:HDL ratio. A small study (n=14) was conducted in patients with familial combined hyperlipidemia, noted for their increased cardiovascular risk due to elevated atherogenic lipoproteins and decreased protective lipoproteins. In a cross-over design, patients were given either 4 g/day of a concentrated fish oil preparation in capsules (Omacor- 44%EPA, 36% DHA as ethyl esters) or placebo (corn oil) for eight weeks. As expected, TG levels were lowered significantly (378 to 210), while HDL cholesterol rose a non-statistical 8%. The relative increase in HDL-2, a more cardioprotective lipid subfraction, was statistically significant. LDL, but not total cholesterol, was significantly increased in the fish oil group. In one group of hyperlipidemic patients, DHA (4 g/day) had a more significant (29%) increase in HDL levels than equivalent levels of EPA. Other clinical trials have also reported that DHA has a slightly more favorable effect on lipid profiles (TG lowering, TG:HDL ratio and lipoprotein fractioning), and post-prandial lipid margination.

It is not uncommon to see elevations in plasma LDL cholesterol after fish oil intake, especially in individuals with elevated triglyceride levels. Since total cholesterol usually remains unchanged in these subjects and it is known that most of the increase is due to an increased shift from VLDL to LDL, the clinical significance of this elevation in plasma LDL cholesterol is not yet known, but LDL subfraction analysis suggests that it is the larger, less-dense (and less atherogenic) LDL fraction which is raised and not the smaller (more atherogenic) LDL particles. One report suggested a potential down-regulation of LDL receptors to account for part of this phenomenon.

In a group of patients (n=64) with chronic renal failure, assigned to either 2.4 g/day fish oil (4 capsules- 3:2 EPA:DHA) or olive oil for 8 weeks; those receiving fish oil had statistically lower TG (21%), higher HDL cholesterol (8%) and no change in total or LDL cholesterol. A small, non-statistical, drop in Lp(a) was seen in these patients but Lp(a) is very rarely measured in other studies and similar drops were not reported in those studies where it was measured. Also, little effect is reported in lowering high sensitivity C-reactive protein (hsCRP), a marker of inflammation and an independent risk factor for cardiovascular disease.

Metabolic Syndrome and Diabetes

Metabolic syndrome is a disorder characterized by insulin resistance, high triglycerides, high LDL and low HDL cholesterol, hypertension and central adiposity. An increasingly prevalent condition considered “pre-diabetic,” individuals with metabolic syndrome are also at an increased risk of cardiovascular disease even before a diabetes diagnosis. In both sucrose and fructose-induced animal models of metabolic syndrome, EPA and DHA from fish oils were able to prevent the onset or diminish several parameters (hypertension, adiposity, dyslipidemias) associated with the syndrome. One animal study concluded that insulin-sensitive GLUT4 activity is enhanced in adipocytes (not myocytes) to account for the fish oil's improvement of insulin sensitivity in these animals. While many of the subjects in the TG lowering trials mentioned previously would likely be categorized as having metabolic syndrome, a trial looking at either the prevention or treatment of individuals by this diagnosis as an end-point has apparently not been performed. In one study of overweight treated hypertensive patients (n=69), likely to be deemed as having metabolic syndrome if lipids were reported, combining fatty fish consumption (dietary) with weight-loss had an additive effect on ambulatory blood pressure and decreased heart rate.

Like those with metabolic syndrome, type 2 diabetic patients are characterized with various lipid disorders, insulin resistance and increased risk for CHD. A cohort
within the Nurses’ Health study (n=5103) who were free of CHD but with diagnosed type 2 diabetes were evaluated for CHD risk, relative to n-3 intake from fish. After adjusting for age and other cardiovascular risk factors, the RRs for CHD were 0.70 (1 to 3 fish meals per month), 0.65 (2 to 4 times per week) and 0.38 (>5 times per week). Fish consumption in this cohort was more protective against CHD by quintile than it was when looking at all the women in the Nurses’ Health Study, implying that n-3 fatty acid supplementation in diabetic patients may prove even more beneficial than in the general population. Consumption of fish is associated with a significantly reduced progression of coronary artery atherosclerosis in women (a higher correlation in diabetic women) with coronary artery disease. Generally, fish and fish oil supplements reduce triglyceride levels and improve HDL levels but seem to have no clinically significant affect on fasting glucose, fasting insulin, HbA_1c, or glucose tolerance tests in diabetic subjects.

In one study, fish protein consumption was associated with a significantly lower risk of microalbuminuria in a nested case-control study of 1150 type 1 diabetic patients, although this lowered risk was also reported in a small group of diabetic patients consuming only concentrated EPA (1.8 g/day). Several animal models have suggested a role for fish oil in general, and DHA specifically, for increasing nerve conduction velocity in diabetic neuropathy. Collectively, these data suggest that diabetic patients should consume 1 to 2 grams per day of n-3 fatty acid from fish, balanced between EPA and DHA.

**Hypertension**

There is a dose-dependent inverse relationship between n-3 fatty acid intake and blood pressure in hypertensive patients, but little effect is noted in normotensive or borderline hypertensives. A meta-analysis of 31 placebo-controlled trials found an average -0.66/-0.35 mm Hg drop in systolic/diastolic blood pressure per gram of n-3 fatty acid consumed in hypertensive patients. Many of these trials used doses in excess of 5 grams per day and were associated with gastrointestinal complaints. Another meta-analysis reported an average reduction of 5.5/3.5 mm Hg in hypertensive patients given at least 3 g/day of n-3 fatty acids. Fish oil consumption (~3.6 g/day from diet) had an additive effect when combined with weight loss in overweight hypertensives (~6.0/-3.0 fish alone, -5.5/-2.2 weight loss alone, -13.0/-9.3 mm Hg combined). The authors conclude that given the magnitude of the BP reduction with the fish/weight loss combination, withdrawal of antihypertensive therapy may have been possible.

DHA and EPA have been tested separately for their hypertensive activities. Mori et al. has reported that 4 g/day of DHA, but not EPA, reduces ambulatory blood pressure and has favorable effects on arterial compliance.

**Additional Cardiovascular Mechanisms**

Discussing the various potential biological mechanisms in detail is beyond the scope of this review. For the sake of those interested in pursuing this avenue, however, a list of reported potential mechanisms attributed to n-3 fatty acids and several references are included below.

- **Anti-inflammatory**
- **Arterial compliance**
- **NO-induced endothelial relaxation**
- **Reduced asymmetric dimethyl arginine (ADMA)**
- **Reducing atherogenic adhesion molecules**
- **Anti-thrombogenic**
- **Stabilizing atherosclerotic plaques**
- **Peroxisome proliferator-activated receptors (PPAR) regulation**

**NON-CARDIOVASCULAR USES**

**Anti-inflammatory- Rheumatic Diseases**

The well-known pathways which convert the 20 carbon n-6 fatty acid arachidonic acid into pro-inflammatory cytokines is often termed the arachidonic acid cascade. Key enzymes in the formation of pro-inflammatory prostaglandins and leukotrienes are the cyclooxygenase (COX) and lipoygenase (LOX) enzymes. Inhibition of these enzymes is one of the most popular anti-inflammatory mechanisms in the pharmaceutical trade. Since the substrate for each of these enzymes is a 20 carbon fatty acid, eicosapentaenoic acid (EPA) is capable of both competing for the use of the enzyme as well as forming eicosanoids which function to counteract the activity of eicosanoids derived from arachidonic acid. These mechanisms have led to the proposal that increasing n-3 (especially EPA from fish) and lowering n-6 fatty acid intake would have a favorable benefit on the overall inflammatory burden, particularly in individuals with chronic conditions such as rheumatoid arthritis.

Omega-3 fatty acids from fish oil have been studied extensively in patients with rheumatoid arthritis. Meta-analysis data suggest a modest improvement in tender joints and morning stiffness with the addition of fish oil supplementation. Dosing and fish oil content vary widely in different clinical trials. The most significant benefits seem to require at least 3 grams/day, although benefits were seen in some trials with 2.6 grams/day, 30 mg/kg/day and 40 mg/kg/day. Significantly more benefit is seen when patients who use fish oil supplements are also consuming a low arachidonic acid, anti-inflammatory diet.

The role of fish oils has also been explored in patients with inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease. Reviews of the various clinical trials have shown that doses as high as 4.5 and 5.4 grams per day have limited benefit on preventing relapses, but often...
reduce the dependence on steroid therapy and dramatically reduce inflammatory markers. A specially prepared enteric-coated, free fatty acid preparation (1.8 g/day EPA, 0.9 g/day DHA) was able to significantly reduce the level of relapse compared to placebo in a group of Crohn’s disease patients (n=78). Another group recently reported that stimulated T-cells and monocytes taken from Crohn’s disease patients supplemented with fish oil (1.6 g/day EPA, 1.08 g/day DHA- non-enteric coated) and an antioxidant blend (Vit. A, C, E, selenium, manganese) produced lower interferon-gamma and PGE2, compared to placebo. In general, these data suggest that individuals with inflammatory bowel conditions may be benefited by increasing fish oil intake equivalent to 2.5-5 grams per day.

Depression and Other Mood Disorders

Long chain n-3 fatty acids are important components of membranes within neurological organs and tissues. They affect membrane fluidity and excitability, influence synaptic function, and perhaps serotonin and dopamine metabolism. In several epidemiological studies, fish consumption is related to decreased risk of depression, especially in women. Although not all cohort studies proved statistically significant, a recent case-controlled study (China) reported that low red blood cell EPA levels are associated with increased risk for attempting suicide. Previous reports suggest there is a link between violent suicides and seasonal intake of EPA.

Several clinical trials have used n-3 fatty acids to treat depression and related disorders. Most of the studies to date have used a preparation of pure EPA (EE form). Peet et al. reported that 1 gram (but not 2 grams) of EPA improved depression scores in patients (n=17 each group) with ongoing medicated depression. However, Nemets et al. reported that similar patients (n=20) receiving 2 grams per day of a comparable preparation had highly significant reduction in Hamilton depression scale scores (mean 12.4 point reduction vs. 1.6 for placebo). This same group attempted to use this preparation at the same dose to treat medicated patients with obsessive compulsive disorder (OCD) without success. Pure DHA (2 g/day) had only a small, non-statistical benefit in patients with major depression. Bipolar patients given high doses of fish oil (6.2 g EPA/3.2 g DHA) had a significantly longer period before relapse than similar patients taking olive oil. Physicians treating patients with depression or related disorders should consider measuring patient serum fatty acid levels and including fish oil supplements (particularly EPA) at 1-2 grams per day.

Maternal and Infant Care

Maternal fatty acid levels, especially DHA levels steadily drop in late pregnancy, increasing risk for post-partum depression. A meta-analysis of 41 studies showed that lower fish consumption and breast milk DHA content were associated with increased risk for post-partum depression. Low doses of DHA (200 mg/day -algae-derived) given post-delivery, however, were unable to significantly lower symptoms of post-partum depression.

The role of n-3 fatty acids in maternal gestation and parturition, as well as offspring development has been reviewed elsewhere. Generally, women with higher n-6 to n-3 intake have a higher likelihood to deliver prematurely. This phenomenon is thought to be related to changes in eicosanoid production (prostaglandins, leukotrienes) which take place prior to parturition. Epidemiological studies suggest that gestation is generally longer in women with higher intake of n-3 fatty acids from fish in some cohorts, but not in others. High n-6 to n-3 fatty ratios also correlate to an increased risk for preeclampsia. Intervention trials, during high risk pregnancies have shown some improvement in prolonging gestation (2.7 g/day n-3), but not in pregnancy related hypertension.

Rapid growth in the brain occurs during the last trimester of pregnancy and the first several postnatal months. The need for maternal DHA is critical during these months since fetal and newborn fatty acid metabolism is inadequate to provide proper levels of DHA for brain development. Several reports suggest that maternal supplementation of fish oils or DHA alone during the third trimester and while breast-feeding can improve cognitive development in newborns, improve sleep patterns (a measure of brain development), and even increase IQ scores at age 4. Maternal fish oil supplementation (3.7 g/day n-3, 56% DHA) in atopic women (offspring considered at high risk for allergic diseases) significantly increased breast milk levels of the protective Immunoglobulin A (IgA) and CD14. Children born from these mothers have reduced levels of allergic related cytokines and allergen-specific immune responses. Children born at high risk for atopic diseases had reduced allergy-related cough at age 3 if they were supplemented with fish oil (500 mg of tuna oil/d- 185 mg n-3) from 6 months to 3 years. Eating high levels of n-3 fatty acids directly from fish is contraindicated in young children and pregnant women due to the potential for ingesting mercury and other toxins. Fish oil supplements, virtually free of these toxins, are safer and allow for specific dosing regimens. Many liquid as well as capsule preparations can be used which provide varying levels of DHA, some of which are specially prepared and flavored for children.

Ocular and Cognitive Health

As a specialized portion of the nervous system, the retina has one of the highest levels of long-chain fatty acids in the human body; especially concentrated is the level of DHA. Infant visual acuity is diminished in n-3 deficiency. Children supplemented with DHA (115 mg/day) from 6 months to 1 year of age had significantly better improved visual acuity than similar control children. The long-term visual benefits for infant supplementation is not
yet known. In adults, fish and DHA intake (determined by food questionnaire) reduces the risk for age-related macular degeneration.\textsuperscript{175,176} Preventative or intervention trials in patients with or at risk for macular degeneration have not been published.

The relationship between DHA and retinitis pigmentosa (RP) is currently being investigated. RP patients have lower levels of DHA,\textsuperscript{177} partly due to reduced activity of the enzyme delta-5-desaturase.\textsuperscript{178} Despite this relationship, trials attempting to slow the progression of RP with supplementation of DHA have been unsuccessful,\textsuperscript{179,180} although chronic vitamin A users who added 1200 mg/d of DHA had some slowing in progression after 2 years.\textsuperscript{181}

Increased dietary intake of fish and DHA (but not EPA) is correlated (cohort of 815) with a decreased risk of Alzheimer’s disease.\textsuperscript{182} Whether this correlation will prove to be of preventative or therapeutic benefit is yet to be determined. Studies also suggest that DHA is protective against dendritic cell damage in a mouse model of Alzheimer’s disease.\textsuperscript{183}

**Fish Oil- The Product**

Recommendations to increase fish consumption are not always straightforward. Some fish have high levels of EPA and DHA; others do not (see chart figure 1). How the fish is prepared also has a significant affect on whether these long-chain fatty acids will be beneficial. In a population-based cohort study, dietary fish consumption was correlated with increased plasma n-3 levels and reduced risk of cardiovascular death in individuals consuming tuna or other similar fish (broiled or baked), but neither was associated with fried fish or fish sandwiches (fish burgers).\textsuperscript{91} The same group reported similar differences for reducing the risk of atrial fibrillations in these different populations based on type of fish consumed.\textsuperscript{92} The susceptibility to loss or modification of EPA and DHA has been reported in various cooking processes, especially deep-frying.\textsuperscript{93} The additional potential hazard of consuming environmental toxins such as methyl mercury and other heavy metals or pesticides like DDT, DDE or PCBs is a concern for many. The Environmental Protection Agency warns those most at risk (pregnant women, nursing mothers and their infants and young children) to limit fish intake to avoid potentially dangerous mercury levels.\textsuperscript{94} Several advantages of fish oil supplements directly address these concerns. Levels of EPA and DHA are consistently dosed in capsule or liquid products. Levels of heavy metals and pesticides can be dramatically reduced, often below detectible limits, when using fish oil supplements in lieu of consuming more fish.\textsuperscript{170,171} Fish oil is inherently more susceptible to oxidation, requiring that most products contain additional fat-soluble antioxidants such as natural vitamin E, fat soluble ascorbates or other natural antioxidants to protect them from becoming rancid under normal storage conditions.

Commercial fish oil is a by-product of the fish meal industry. It is typically a blend of many different fish species including mackerel, anchovies, sardines, tuna, salmon and others. The raw oil from these fish is then purified and concentrated by removing (hydrolyzing) the individual fatty acids from the fish triglycerides so the various fatty acids can be separated and concentrated. This process allows for the separation of contaminant toxins, proteins (which may increase allergenicity and burping), and other non n-3 fatty acids. These concentrated fatty acids remain as free fatty acids (FFA) before they are stabilized by esterification to ethanol (ethyl esters, EE) or further esterified back to a glycerol backbone to create a re-esterified triglyceride (rTG). Both EE and rTG forms of varying concentration (30-70% EPA+DHA) are used in the dietary supplement industry throughout the United States.

Few studies have looked at differences between fish oil supplements provided as EE or rTG. One study reported that plasma EPA and DHA levels were higher when equivalent levels of these fatty acids were consumed directly from salmon than from fish oil supplements provided as ethyl esters.\textsuperscript{95} Whether the ethyl ester form diminished or some fish component enhanced bioavailability is not known. Several studies have shown that plasma bioavailability of the EE form is less than 50% of that from the rTG form.\textsuperscript{96,97,98} Other studies, however, show no difference in bioavailability between these two forms.\textsuperscript{99,100} All of these studies were uncontrolled and involved very few subjects. Dyerberg et al.\textsuperscript{114} completed a study involving 72 subjects, comparing the bioavailability of EPA and DHA from natural fish triglycerides, EE, rTG, cod liver oil and FFA. They found that compared to natural fish TG (100% standard), the bioavailability of EPA and DHA combined was highest from the re-esterified TG (124%) and lowest from EE (73%). The EPA and DHA incorporated into phospholipids was 62% and 290% greater when consumed as rTG rather than EE. At this time there are no trials comparing the potential differences in the EE and rTG forms as it pertains to clinical outcomes (triaclylglyceride lowering, hypertension, etc.); many reports don’t specify the forms used. Since data suggests that individuals are likely to absorb the rTG form better, and lipase and biological incorporation of the EE is diminished,\textsuperscript{99,101} clinical trials should be done to assess whether the rTG form may have better clinical outcomes, or require lower doses for equivalent results. Consistent results at a lower dose would help increase compliance and reduce both side-effects and cost.

As with any dietary supplement, choosing a high-quality fish oil product is important. The Council for Responsible Nutrition (CRN), along with many of the leading fish oil manufacturers in the world, published a monograph in 2002 outlining various quality aspects which the industry should use to regulate fish oil products.\textsuperscript{102} This monograph stipulates upper limits for mercury and other...
heavy metals, pesticide levels and oxidation levels such as peroxide and anisidine values. These guidelines have been adopted by the United States Pharmacopoeia (USP) for their current n-3 fatty acid from fish oil monograph.\textsuperscript{103} Additionally, some companies monitor the production from catch to finished product in order to provide kosher products to the market.

**Side-effects and Contraindications**

High-dose fish oil supplementation is extremely well tolerated in nearly all individuals. The most common side-effect is a fishy aftertaste or “burping” associated with high doses. When products are consumed with meals and carbonated beverages are avoided, this unpleasant feature is dramatically reduced. The complete purification of the fatty acids from fish proteins virtually eliminates the potential for allergic components in the fish oil supplements. Because fish oil is prone to oxidation, consuming high doses without additional antioxidant protection (from diet or supplemental sources) may increase vulnerability to lipid peroxidation, especially in warm and sunny climates. While the in vivo consequences of this vulnerability are still being debated, antioxidant supplementation should be recommended for every individual consuming high amounts (3 grams or more) of fish oil daily. These high doses can be consumed directly from bottles for those wanting to avoid gelatin capsules due to concerns about consuming non-fish animals in general or bovine-derived products specifically.

The most frequent contraindication concern is the combination of high dose fish oil with pharmaceutical drugs that affect blood clotting (coumadin, aspirin, etc.), used by many cardiovascular patients. A group of 250 patients who had undergone coronary artery bypass surgery were given 4 g/day of fish oil concentrate and either aspirin (300 mg/d) or warfarin therapy.\textsuperscript{111} Compared to patients not receiving fish oil, these patients had no increase in bleeding time. Another report showed no change in INR when 6 g/day of fish oil was given to patients on chronic warfarin therapy.\textsuperscript{112} However, one case report has been published of a woman (67 years old on coumadin, 1.5 years at 1.5 mg/day) who had an increased INR (2.8 to 4.3) in the month she doubled her fish oil supplement from 1 to 2 g/day.\textsuperscript{113} These data suggest that the concern for bleeding times is generally not an issue, but INR should be checked in patients on both warfarin and fish oil therapies.

**CONCLUSION**

Epidemiological evidence is quite clear in demonstrating numerous health benefits in consuming long-chain polyunsaturated n-3 fatty acid from fish, especially as a ratio to n-6 fatty acids derived from vegetable oils. Even an “Omega-3 Index” of RBC EPA and DHA levels is being proposed as a routine laboratory test for measuring cardiovascular risk.\textsuperscript{109} In the past decade, the clinical use of fish oil supplements has greatly increased, as has the data supporting their use. While dietary and lifestyle changes are ideal ways to modify a number of cardiovascular risk factors, many individuals with personal or family history of cardiovascular disease cannot safely consume high levels of n-3 fatty acids from fish alone, or do not maintain the dietary habit.\textsuperscript{24} Since fish oil supplements have been shown to have beneficial effects on nearly every risk factor for cardiovascular disease, and so many individuals are currently at risk, the recommendation to use these supplements in clinical practice is encouraged. There are few patients who would not realize some benefit by increasing their fatty fish consumption or adding fish oil supplements to their daily routine. Patients with previous CHD, hypertriglyceridemia, hypertension, type II diabetes or metabolic syndrome should be taking at least 2 g/day of n-3 fatty acids from fish oil daily via supplements. Pregnant women should be encouraged to consume fish oil supplements to increase n-3 fatty acids, particularly DHA throughout the second half of pregnancy and while breast-feeding.

**Disclosure statement:** This author is affiliated with a company, Ortho Molecular Products, that manufactures and distributes dietary supplements, including fish oil products.

**REFERENCES**


103. USP Website http://www.uspverified.org/standards/monographs.html


While the value of Omega-3 fish oil supplementation is widely known, their quality and potency can vary significantly.

Potency. Ortho Molecular is known as a leader in efficacy. Our burp-free OrthoMega triglyceride oils are among the highest dosage available, providing your patients with the easiest compliance in the industry. Most other products require patients to take up to four times as much to equal a single dose of OrthoMega.

Purity. Ortho Molecular Products is one of a handful of companies whose fish oil capsules comply with the CRN Monograph for quality. Our entire line of OrthoMega fish oils is tested free of pesticides, heavy metals and contaminants.

OrthoMega fish oils are the most efficacious professional fish oil supplements available. Demand the best: try OrthoMega today!