

December 23, 2003

PETITIONER

Belovo, Inc., Pinehurst, N.C. 28374

On behalf of:

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Subject: Petition for a qualified health claim for enhanced omega-3 fatty acid in eggs with a balanced ratio of omega-6/omega-3 fatty acids

Petition submitted to:

Food and Drug Administration

Office of Nutritional Products, Labeling and Dietary Supplements

HFS-800

5100 Paint Branch Parkway

College Park, MD 20740

INTRODUCTION AND PURPOSE

The undersigned, Belovo Inc., submits this qualified health claim petition pursuant to section 403(r)(4) or 403(r)(5)(D) of the Federal Food, Drug, and Cosmetic Act, and in accordance with the guidelines of the Task Force on the Consumer Health Information for Better Nutrition, with respect to eggs containing an increased amount of omega-3 fatty acids with a balanced ratio of omega-3/omega-6 fatty acids and their relationship to reducing the risk of certain cardiovascular diseases. Attached hereto, and constituting a part of this petition, are the following sections and information required by 21 CFR §101.70(f).

On December 18, 2002, FDA launched the "Consumer Health Information for Better Nutrition" initiative and stated that it would develop a process to allow qualified health claims (QHC) on both conventional foods and dietary supplements. A Task Force was established to develop recommendations for implementing the QHC process. In July 2003, FDA issued recommendations provided by the Task Force on Consumer Health

Information for Better Nutrition for implementation of this new program beginning September 2003. The intent of this initiative is to help consumers make informed choices about their diet and nutrition in order to improve the overall health of the American public. The stated goals of the FDA initiative are to 1) allow for more understandable and science-based information in labeling of foods and nutritional supplements; 2) to better inform the consumer on how dietary choices affect their health and 3) encourage companies to compete based on the health and nutritional consequences of their ingredients, in addition to non-health related features like taste and convenience.

The petitioner, Belovo, Inc., believes that the FDA's qualified health claim program is a positive step, which can greatly benefit the health of consumers in making informed decisions regarding their diet. The petitioner is requesting that FDA allow for a qualified health claim as given in the model claim (Section B (10)) for reduction of risk of heart disease and sudden cardiac arrest from ingestion of a conventional food, namely eggs, containing enhanced levels of omega-3 fatty acids that are balanced in approximately a 1:1 ratio with omega-6 fatty acids. In its announcement of the QHC program, FDA specifically mentions the benefits of eating several servings of foods high in omega-3 fatty acids in reducing the risk of heart disease. Belovo SA (Belgium) has developed the technology for producing eggs, a common food in the diet, containing increased levels of omega 3 fatty acids which achieve the desired nutritional and health benefits. This egg, which is the subject of this petition typically contains 660 mg of omega-3 fatty acids per 50-g edible portion of which 550 mg is ALA, 10 mg EPA, 20 mg DPA, and 80 mg DHA. The egg is currently being marketed in the U.S. as the Christopher[®] egg and in Europe as the Columbus[®] egg. As a point of reference, a standard 50g egg contains 37.5mg of omega-3 fatty acids (www.aeb.org; www.nal.usda.gov/fnic/cgi-bin/nut_search.pl).

This petition has been prepared according to the requirements for health claim petitions in 21 CFR §101.70 and the guidance documents for qualified health claims provided by the Task Force on Consumer Health Information for Better Nutrition in July 2003 (<http://www.fda.gov/oc/mcclellan/chbn.html>). As part of this petition, the petitioner

is presenting a comprehensive evaluation of the scientific basis for its claim including a biological mechanism by which omega-3 fatty acids reduce cardiovascular disease, the rationale for preferentially using a 1:1 ratio of omega-3/omega-6 in the diet and other scientific reports and human clinical and epidemiological studies on the relationship between intake of polyunsaturated fats and/or cholesterol and cardiovascular diseases such as atherosclerosis, coronary heart disease, stroke, sudden cardiac arrest and arrhythmia. We believe that the petition presents far more than just credible evidence that the scientific data support the proposed claim. As this petition also shows, there is a general acceptance by the scientific community and, increasingly, by various expert governmental bodies and private institutions, that an increased intake of omega-3 fatty acids decreases the risk of cardiovascular disease. The scientific evaluation presented herein and the model claim have been reviewed and approved by consensus of an expert panel qualified to evaluate the scientific evidence. The signatures of the expert panel agreeing to the summary consensus statement, as well as attached curriculum vitae, are included as part of this petition.

A) FDA PRELIMINARY REQUIREMENTS FOR HEALTH CLAIMS

In order for a substance to be eligible for a health claim, it must meet the eligibility requirements of 21 CFR §101.14 as follows:

21 CFR §101.14 (b) Eligibility. For a substance to be eligible for a health claim:

- (1) The substance must be associated with a disease or health-related condition for which the general U.S. population, or an identified U.S. population subgroup (e.g., the elderly) is at risk, or, alternatively, the petition submitted by the proponent of the claim otherwise explains the prevalence of the disease or health-related condition in the U.S. population and the relevance of the claim in the context of the total daily diet and satisfies the other requirements of this section.
- (2) If the substance is to be consumed as a component of a conventional food at decreased dietary levels, the substance must be a nutrient listed in 21 U.S.C. 343(q)(1)(C) or (q)(1)(D), or one that the Food and Drug Administration (FDA) has required to be included in the label or labeling under 21 U.S.C. 343(q)(2)(A); or
- (3) If the substance is to be consumed at other than decreased dietary levels:
 - (i) The substance must, regardless of whether the food is a conventional food or a dietary supplement, contribute taste, aroma, or nutritive value, or any other technical effect listed in

Sec. 170.3(o) of this chapter, to the food and must retain that attribute when consumed at levels that are necessary to justify a claim; and

(ii) The substance must be a food or a food ingredient or a component of a food ingredient whose use at the levels necessary to justify a claim has been demonstrated by the proponent of the claim, to FDA's satisfaction, to be safe and lawful under the applicable food safety provisions of the Federal Food, Drug, and Cosmetic

21 CFR §101.14(e) Prohibited health claims. No expressed or implied health claim may be made on the label or in labeling for a food, regardless of whether the food is in conventional food form or dietary supplement form, unless:

* * *

(3) None of the disqualifying levels is exceeded in the food...unless FDA has permitted a claim despite the fact that a disqualifying level of a nutrient is present in the food based on a finding that such claim will assist consumers in maintaining healthy dietary practices, and...the label bears a disclosure statement that complies with 101.13(h), highlighting the nutrient that exceeds the disqualifying level.

* * *

(6) Except for dietary supplements or where provided for in other regulations in part 101, subpart E, the food contains 10 percent or more of the Reference Daily Intake or the Daily Reference Value for vitamin A, vitamin C, iron, calcium, protein, or fiber per reference amount customarily consumed prior to any nutrient addition.

In the case of an egg, which contains greater than 60 mg of cholesterol per serving (typical single egg with 50 g content), an exception from disqualifying nutrient levels for cholesterol in 21 CFR §101.14(a)(4), as noted below, is required.

(4) Disqualifying nutrient levels means the levels of total fat, saturated fat, cholesterol, or sodium in a food above which the food will be disqualified from making a health claim. These levels are 13.0 grams (g) of fat, 4.0 g of saturated fat, 60 milligrams (mg) of cholesterol, or 480 mg of sodium, per reference amount customarily consumed, per label serving size, and, only for foods with reference amounts customarily consumed of 30 g or less or 2 tablespoons or less, per 50 g. For dehydrated foods that must have water added to them prior to typical consumption, the per 50-g criterion refers to the as prepared form. Any one of the levels, on a per reference amount customarily consumed, a per label serving size or, when applicable, a per 50 g basis, will disqualify a food from making a health claim unless an exception is provided in subpart E of this part, etc.

1) Omega-3 fatty acids meet the definition of substance in 21 CFR §101.14(a).

Omega-3 fatty acids in eggs, which are the subject of this claim, meet the definition of a substance under 21 CFR §101.14(a)(2), “ a specific food or component of food, regardless of whether the food is in conventional food form or a dietary supplement that includes vitamins, minerals, herbs or other similar nutritional substances.” Omega-3 fatty acids are among the polyunsaturated fatty acids that are considered essential fatty acids, which must be obtained from the diet. Omega-3 fatty acids are naturally found at relatively high levels in fish, flax, hemp, rape (canola) seed, soybean, and in walnut, chia and perilla oils. The egg that is the subject of this petition typically contains 660 mg of omega-3 fatty acids per 50-g edible portion of which 550 mg is ALA, 10 mg EPA, 20 mg DPA, and 80 mg DHA. The eggs have enhanced amounts of these omega-3 fatty acids by means of feeding the laying chickens a diet of natural plant sources such as flaxseed high in omega-3 fatty acids. Therefore, the omega-3 fatty acids are defined components of a conventional food, namely eggs, and meet the FDA definition of a substance under 21 CFR §101.14(a)(2).

2) 21 CFR §101.14(b)(1) Omega-3 fatty acids and a balanced intake of omega-3/omega-6 fatty acids are associated with reduction in diseases affecting the general U.S. population

The America Heart Association (AHA), working in concert with the Centers for Disease Control National Center for Health Statistics, the National Heart, Lung, and Blood Institute and other government agencies, publishes an annual summary of cardiovascular disease statistics and has released its 2003 update based on statistics from 2000 (American Heart Association, 2003)². The major disease entities defining cardiovascular disease and US population affected are: hypertension (50 million); coronary heart disease including myocardial infarction (heart attack) (7.6 million) or angina pectoris (chest pain) (6.6 million); congestive heart failure (4.9 million); stroke (4.7 million) and congenital defects (1 million). The AHA estimates that approximately 62 million Americans or 1 in 5 men and women have some form of cardiovascular disease. Thus, cardiovascular disease is by far the leading cause of mortality and illness in the United States. In 2000, nearly 40% of all deaths were associated with cardiovascular disease (946,000/year or 2600/day). Cardiovascular disease causes more deaths than the next five leading causes including cancer, respiratory disease, accidents, diabetes and influenza.

Several additional factors indicate that illness and death from cardiovascular disease will increase in the future. The aging of the population, alarming increases in obesity and type-2 diabetes and associated hyperlipidemia, atherosclerosis and hypertension as well as concerns about diet and obesity in younger generations have led the AHA and health-related governmental agencies to consider cardiovascular disease a problem of epidemic consequences, now and in the future. In addition to the human toll associated with cardiovascular disease, the economic costs are enormous. Direct health care costs for cardiovascular disease were estimated to be over \$200 billion and indirect costs from premature mortality or illness in lost productivity is over \$140 billion in 2003. The majority of these costs (\$360 billion) were associated with coronary and other heart diseases.

The risk factors for cardiovascular diseases in descending order of importance are: tobacco smoking; diet and nutrition; hyperlipidemia and hypercholesterolemia; overweight or obese condition and associated metabolic syndrome (estimated at 47 million US residents); diabetes mellitus (7.3% of US population); sedentary lifestyles and quality of medical care including smoking cessation, blood pressure and cholesterol control and screening and use of beta-blockers after a heart attack. With the exception of smoking, all of the underlying risk factors for cardiovascular disease are directly and/or indirectly associated with diet and nutrition.

As the above summary clearly demonstrates, the most significant impact on the prevalence of cardiovascular disease in the US is likely to be from improvements in dietary and nutritional habits. However, despite years of public health and medical messages and advisories to the public regarding the health problems associated with excessive caloric intake, lack of exercise and diets high in fats and sugars, the great majority of Americans consumed 300 calories more in 2000 than in 1985, mostly in refined grains or carbohydrates, added fats and added sugars (Putnam *et al.*, 2002)².

Why do Americans continue to make such poor dietary choices and fail to heed sensible public health advice regarding our diet? Dietary choices are strongly influenced by cultural and economic factors, as well as the choices presented to the consumer from modern agricultural and food processing practices. Obviously, taste and satiety are primary determinants in the types and amounts of food ingested. For some consumers, health and nutritional values of foods are very important, but, for most consumers, these rank behind taste, cost and convenience as purchasing criteria. In affluent societies where cost of food is usually not a limiting factor, consumers are able to purchase high fat-containing foods such as red meats and highly processed ready-to-eat foods, which often contain sugars, hydrogenated vegetable oils and saturated fats. Hydrogenated fats from vegetable oils are commonly added to many foods such as baked goods and fried foods without the consumer's awareness. Due to convenience and taste, the use of high fructose corn syrup in soft drinks has become a major source of added dietary sugars. Furthermore, in a modern agriculture dominated by cereal grains such as wheat, corn, rice and maize, it is not surprising that the most prevalent and economic foods in the market are based on these commodities.

In contrast to these negative dietary trends, there is an increased interest in the health benefits of foods and nutritional supplements. One promising approach to improving consumer choices toward healthier foods and reducing cardiovascular disease is to incorporate the desired substances into conventional foods that are commonly eaten and to fully inform the consumer of the benefits by health claim labeling of these foods. In this case, even if the consumers did not markedly change their diet, normal egg consumption, if totally replaced with an omega-3 enriched egg, would confer a health benefit in reduction of cardiovascular disease risk. In this manner, it is possible to deliver physiologically important and beneficial nutrients into the daily diet without having to overcome longstanding cultural food preferences in the general population.

3) 21 CFR §101.14(b)(3)(i) Omega-3 fatty acids and a balanced intake of omega-3/omega-6 fatty acids contribute and retain nutritive value in conventional foods, such as when consumed at 1-2 omega 3-enhanced eggs/day

Two polyunsaturated fatty acids, which include naturally occurring omega-3 alpha-linolenic acid (ALA) and omega-6 linoleic acid (LA), are termed “essential fatty acids (EFAs).” As such, ALA and LA cannot be synthesized by humans and must be obtained from the diet. In the body, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) can be made from ALA, and arachidonic acid (AA) can be made from LA, but these pathways compete against each other and are not always fully operational. For this reason, EPA, DPA, DHA and AA are termed “conditionally essential fatty acids” and referred to as long chain polyunsaturated fatty acids (LCPUFAs). Vegetable oils, such as corn oil, cottonseed oil, soybean oil and flaxseed oil are sources of LA and ALA. Animal fats, such as fish oils and meat fats are sources of EPA, DPA, DHA and AA.

EFAs and LCPUFAs are needed for many physiologic processes, including maintaining the integrity of the skin, the structure of cell membranes and in the synthesis of prostaglandins and leukotrienes. Docosahexaenoic acid is an important component of the brain and retina. Full-term babies fed a skim-milk formula low in linoleic acid may have growth failure, thrombocytopenia, alopecia, and a generalized scaly dermatitis, with increased water loss from the skin. Essential fatty acid deficiency in adults may result in scaly skin, infertility and neurological effects.

In recognition of the essential nutritive value of omega-6 and omega-3 fatty acids, the USDA has recently proposed that they be included in the listing of nutritional components of the Food Guide Pyramid (68 FR 53536, September 11, 2003)³. In Table 3 of this notice, the nutritional goals for proposed daily food intake patterns include specific linoleic acid and alpha-linolenic acid intake for the several levels of daily caloric intake. For example, Adequate Intakes (AI) of linoleic acid and alpha-linolenic acid for a 2400 calorie diet are 17 and 1.6 g/day, respectively and the Acceptable Macronutrient Distribution Ranges (AMDR) are 5-10% and 0.6-1.2% of total caloric intake for linoleic acid and alpha-linolenic acid.

4) 21 CFR §101.14(b)(3)(ii) Omega-3 fatty acids and a balanced intake of omega-3/omega-6 fatty acids are safe and lawful under the FDCA

The eligibility for classification of a food component or food ingredient as Generally Recognized As Safe (GRAS) by FDA is presented in 21 CFR §170.30. With regard to essential fatty acids such as omega-3 and omega-6 fatty acids that are the subject of this petition, they would fall within the GRAS criteria in 21 CFR §170.30(c)(1) as a food component of natural biological origin commonly present in food prior to January 1, 1958.

Furthermore, FDA has affirmed the GRAS status of menhaden oil under 21 CFR §184.1472, which contains approximately 12 g EPA/100g oil and 8 g DHA/100g oil. FDA had no questions on the GRAS notification of tuna oil (GRN 000109) containing 6 g EPA/100g oil and 26.5 g DHA/100g oil. FDA also had no questions on the GRAS notification of fish oil concentrate (GRN 000105) for specified uses of 20 g EPA/100g and 18 g DHA/100g oil concentrate. In both of the Agency response letters to the GRAS notices, FDA advised that the intake of EPA and DHA should not exceed 3 g/p/day due to possibly increased bleeding time, increased levels of low-density lipoprotein cholesterol, and effects on glycemic control in non-insulin dependent diabetics. The egg that is the subject of this petition typically contains, per 100-g edible egg, 1320 mg of omega-3 fatty acids of which 1100 mg is ALA (potentially convertible to LCPUFAs in the body) and 220 mg LCPUFAs, among which are 20 mg EPA, 40 mg DPA and 160 mg DHA. Therefore, consumption of 2 eggs/day would result in a maximum total intake of ALA, EPA, DPA and DHA equivalents of approximately 1320 mg/day, well below the 3 g/day considered an upper limit of intake for addition to foods of the fish oils.

Given the potential of omega-3 fatty acids to modulate the formation and intensity of eicosanoid products (Section B. (3) below), excessive intakes of omega-3 fatty acids (>5-10 g/day) may behave as cyclooxygenase (COX-1 and COX-2) inhibitors, such as aspirin and non-steroidal anti-inflammatory drugs, to increase clotting times, susceptibility to bruises, nosebleeds, hematuria and gastrointestinal bleeding. The safety of omega-3 fatty acids is also addressed in a recent American Heart Association Scientific Statement (Kris-Etherton et al., 2002)⁴. The AHA position reiterates the FDA ruling that intakes of up to 3 g/day of marine omega-3 fatty acids are GRAS (Generally

Recognized As Safe) for inclusion in the diet. This ruling included specific consideration for reported effects of omega-3 fatty acids on glycemic control in patients with diabetes, on bleeding tendencies, and on LDL-cholesterol. The risk for side effects from ingesting up to 3 g/day of omega-3 fatty acids is very low for clinical bleeding, low for worsening glycemia and moderate for a rise in LDL-cholesterol and usually this occurs only in patients with hypertriglyceridemia.

Several prospective cohort studies have indicated that intake of omega-3 fatty acids at levels as low as 200 mg/day reduces the risk of cardiovascular disease such as myocardial reinfarction, sudden cardiac death and overall mortality from coronary heart disease. The levels of omega-3 fatty acids in the enhanced eggs in this petition of 1320 mg omega-3 fatty acids/100-g edible egg (or 220 mg LCPUFAs per 100 g edible egg) are sufficient to provide an intake that would be of health benefit, thus justifying a qualified health claim as well as being an acceptably safe level.

In its letter regarding use of a qualified health claim for omega-3 fatty acids on dietary supplements (Docket No 91N-0103) dated October 31, 2000, FDA addressed consumer health and safety from exposure to the omega-3 fatty acids EPA and DHA with a focus on their use and consumption from dietary supplements and the consequent addition to the overall dietary intake of omega-3 fatty acids. FDA expressed concern that intake from dietary supplements would be much greater than that from foods estimated in the GRAS determinations. The agency determined that it was necessary to limit exposure to EPA and DHA from dietary supplements and allowed the qualified health claim only for dietary supplements that recommended a maximum daily intake of no more than 2g/day from such a supplement. This limit was established on the basis of estimates of current intakes of foods that naturally contain EPA and DHA and of other food sources of EPA and DHA.

The omega-3 fatty acid enhanced egg that is the subject of this petition is well within the exposure range that FDA permitted for dietary supplements (total EPA and DHA per 50 gram serving is 90 mg). While the consumption of the omega-3 enhanced egg in combination with other food sources including fish and dietary supplements may collectively increase exposure to EPA and DHA, the total intake will still be below the 3g/p/day established by FDA as the maximum daily exposure. Based on the scientific

data cited in FDA's letter referenced above, as well as the information presented in Section B of this petition, an additional increase of omega-3 fatty acids in the total diet from foods is actually desirable. Further, it is highly unlikely that individuals consciously consuming the omega-3 fatty acid enhanced eggs for their omega-3 fatty acid content of EPA and DHA would also take dietary supplements containing these omega-3 fatty acids. Therefore, the levels proposed in this petition for enhancement of the omega-3 fatty acid content in eggs are safe and lawful under FFDCA and suitable for a qualified health claim.

In summary, the enhancement of eggs with omega-3 fatty acids with a balanced 1:1 ratio of omega-3 to omega-6 fatty acids meets all of the eligibility requirements of 21 CFR §101.14(b); therefore, the preliminary requirements of 21 CFR §101.70 for a qualified health claim petition are acceptably met.

5) 21 CFR §101.14(e)(3) Disqualifying nutrient levels: Request for an exception from the disqualifying nutrient restriction of qualified health claims for omega-3 enhanced eggs containing cholesterol

In the report provided by the Task Force on Consumer Health Information for Better Nutrition (Attachment A-Possible Regulatory Frameworks for Qualified Health Claims), the Task Force noted the existing regulation on disqualifying nutrient levels (21 CFR §101.14(a)(4)) and prior exceptions granted to this rule when there is a public health benefit (e.g. stanol and sterol esters in table spreads). The Task Force recommended that FDA remain flexible and open to appropriate exceptions to this regulation. We agree that the FDA should offer flexibility in this area and evaluate exceptions as requested on a case-by-case basis in order to allow a greater range of food producers to develop healthier foods and gain appropriate health claims. We believe the FDA should consider emerging science and new understandings of nutritional factors in determining whether these fixed restrictions are outdated and whether other considerations, such as overriding health benefits from the overall food composition, should be determinative for proper labeling and health claims.

As the following discussion details, the application of an exception to disqualifying nutrient levels is well justified for an omega-3 fatty acid enhanced egg. As a natural food with high lipid content, there are no whole eggs on the market, nor are there likely to be, whole unprocessed eggs with less than 60 mg cholesterol/egg or serving as prescribed by the regulation. In theory, any fat-soluble nutrient could be introduced into the diet in a common food (eggs) by fortifying the diet of the laying hens. Therefore, strict application of this regulation would effectively prevent egg producers from informing the public about any improvements in their products that would have health benefits and would also remove health-based incentives for product improvement.

Eggs are a highly nutritious food (www.enc-online.org/; www.aeb.org). One large egg provides approximately six grams of protein, about half of which is in the egg yolk. Egg white is considered an ideal protein and serves as a reference standard to which all other protein sources are compared because it contains all the essential amino acids in the proper proportion required for human nutrition. A standard egg provides 10 percent of the daily value of protein based on a 2,000 calorie diet. The only food that contains a more ideal mix of essential amino acids than an egg is mother's breast milk.

Eggs are also significant sources of iron, riboflavin, folate, vitamins B-12, D and E. The iron in egg yolks, like the iron in meat is highly bioavailable. An egg also contributes 13 vitamins and minerals in varying amounts and is low in sodium (65 mg). Two large eggs represent only 7 percent of the total daily caloric intake of a person on a 2,000 calorie diet and provide 20 percent of the daily value for protein, 30 percent of the daily value for riboflavin, and 8 percent or more of the daily value of several nutrients including vitamins A, D, E, B-6, B-12, folate, iron, phosphorus, and zinc.

In the Food Guide Pyramid (<http://www.usda.gov/cnpp/DietGd.pdf>), eggs are a part of the group of protein-rich foods, officially known as the "Meat, Poultry, Fish, Dry Beans, Eggs and Nuts Group." The Pyramid calls for two to three servings from this group every day, for a total of five to seven ounces of meat or its equivalent. One egg can

be substituted for one ounce of meat and counts as one-third to one-half of a meat group serving.

A standard egg also provides choline (280mg) and meets more than half of the human's daily need for this nutrient. Eggs are also an important source of the carotenoids lutein and zeaxanthin, that have been shown to have beneficial effects on age related macular degeneration. Of the total fat (4.5 g) in a standard egg, more than half is unsaturated. These unsaturated fatty acids include the physiologically important omega-3 and omega-6 fatty acids (www.enc-online.org).

For the consumer, the egg is an inexpensive, delicious, easy to prepare and digest food that is not only convenient to consume but also provides a very important source of positive nutrients. The omega-3 enriched eggs that are the subject of this petition not only provide the nutrients expected from a standard egg, but will also provide a clear public health benefit from an easily accessible dietary source of omega-3 fatty acids whose consumption has been shown to have a positive impact on the reduction of coronary heart disease.

The relationship between dietary cholesterol, egg consumption and coronary heart disease has recently been reviewed by Hu *et al.*, (2001)⁵. The review shows that the type of fat, not the total amount of fat, is the best predictor of serum cholesterol levels. In metabolic studies, dietary cholesterol has been shown to raise levels of total and LDL cholesterol in blood, but the effects are considered by these investigators to be relatively small compared with saturated and *trans* fatty acids. As stated in this review, there is little direct evidence linking higher egg consumption and increased risk of CHD. In the Framingham study, Dawber *et al.*, (1982)⁶ found no significant association between egg consumption and incidence of CHD despite a wide range of egg intake. In an earlier analysis of the Seventh-Day Adventists study, higher egg consumption appeared to be associated with increased risk of fatal CHD, but this association was not present in a more recent analysis with a longer follow-up (Fraser, 1994)⁷. In a case-control study conducted in Italy, the frequency of egg consumption was not significantly associated with risk of

CHD in women (Gramenzi *et al.*, 1990)⁸. In an analysis of egg consumption and incidence of CHD among 117,933 apparently healthy subjects in the Nurses' Health Study and Health Professionals' Follow-up Study, Hu and colleagues (1999)⁹ found no evidence of an overall positive association between egg consumption and risk of CHD in either men or women. The relative risks (RRs) of CHD across categories of intake (<1/week, 1/wk, 2-4/week, 5-6/week, >=1/day) were 1.0, 1.06, 1.12, 0.90, and 1.08 (*p* for trend=0.75) in men and 1.0, 0.82, 0.99, 0.95, and 0.82 (*p* for trend=0.95) in women. Differences in non-egg cholesterol intake did not appear to be responsible for the lack of an association between egg consumption and risk of CHD.

Nearly two thirds of the cholesterol in the body is synthesized *de novo*, primarily in the liver and intestinal tract. The synthesis and utilization of cholesterol is tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. Normal adults make approximately 1 g of cholesterol/day and ingest about 0.3 g/day. It appears that serum cholesterol response to added dietary cholesterol is modulated by baseline cholesterol intake. The higher the baseline cholesterol intake, the less the response induced by adding cholesterol to the diet. In an analysis of 224 published studies, Howell *et al.* (1997)¹⁰ estimated that a reduction of 100 mg/day of cholesterol in the diet would only result in a 2.2 mg/dl decrease in serum cholesterol. This model was supported by other predictive equations published since 1990, all of which have a narrow range of serum cholesterol reduction (1.37-2.68 mg/dl) predicted for a 100 mg/day decrease in dietary cholesterol. A decrease of 2.2 mg/dl would represent approximately a 1% decrease in average serum cholesterol levels in the U.S. adult population.

A review of epidemiology studies which investigated dietary cholesterol and eggs and their relationship to coronary heart disease concluded that, when other dietary confounders were considered, daily egg consumption of 1 plus eggs was not associated with an increased risk of coronary heart disease (Kritchevsky and Kritchevsky, 2000)¹¹. The overall increased risk of coronary heart disease was small after considering other factors, about 6% per 200 mg cholesterol intake per 1000 calories of daily dietary intake.

In contrast to these findings, Weggemans *et al.*, (2001)¹² evaluated seventeen dietary studies of egg intake and cholesterol and other blood lipid profiles and found that a 200 mg/day intake of cholesterol increased the ratio of total to HDL cholesterol by 0.040 units, which, considering this factor alone, may result in an increased risk of myocardial infarction of 2.1%. However, these studies generally evaluated the only the classical HDL cholesterol (HDL2 and HDL3) and did not measure the more important dietary cholesterol-mediated HDL (HDL1 and HDLc).

Jiang and Sim (1993)¹³ have investigated the effects of omega-3 enriched eggs on the lipid status of young healthy males. Subjects given 2 enriched eggs/day for 18 days showed several beneficial changes in their lipid profile in comparison to controls ingesting 2 standard eggs/day. HDL cholesterol was significantly increased and plasma triglycerides significantly decreased by omega-3 enriched egg intake. Plasma total cholesterol and LDL-cholesterol were increased in controls eating 2 eggs/day, but were essentially unchanged by intake of 2 standard eggs with a total of 1170 mg omega-3 fatty acids. Plasma fatty acid composition of subjects ingesting 2 enriched eggs/day also showed a moderate increase in omega-3 PUFA content.

Two studies have evaluated the effects of substituting Columbus[®] eggs for standard eggs. In post-menopausal Spanish women, intake of 1.13 Columbus[®] eggs, rather than 0.65 standard eggs, per day for eight weeks had no effect on total cholesterol, LDL-or HDL-cholesterol. Serum triglycerides were significantly reduced by 9.6% (Prado-Martinez *et al.*, 2003)¹⁴ In another study (Watrin *et al.*, 2003)¹⁵ of hypercholesteremic adolescents who ate 4 Columbus[®] eggs/week for eight weeks, the results were somewhat variable within the group, but there was a mean 18% reduction in triglycerides, slight decrease in total cholesterol, and essentially no change in HDL or LDL cholesterol levels.

The typical Columbus[®] or Christopher[®] egg contains 175 ± 25 mg/ cholesterol, which is over 10-20% less cholesterol than a standard egg containing approximately 220 mg cholesterol. The American Heart Association (2000), in its dietary guidelines,

recommended that daily intake of cholesterol from all sources be below 300 mg/day, on average, from all dietary sources for the general population. AHA noted the results of Hu et al (1999) that intake of up to one standard egg/day was not associated with increased risk of coronary heart disease in large scale studies. Therefore, consumption of one Columbus® or Christopher® egg/day would be well within the recommended limits on cholesterol consumption by AHA as part of a low-fat dietary regimen.

In summary, the scientific consensus is that the type of fat consumed, rather than the total amount, is the critical factor in resulting serum lipid profiles and coronary heart disease risk. Saturated fat is the primary determinant of LDL cholesterol levels. Trans-fatty acids increase LDL cholesterol and decrease HDL cholesterol. Monounsaturated and polyunsaturated fat replacement of saturated fats is associated with improvements in lipid profiles with lower LDL cholesterol, higher HDL cholesterol and lowered triglycerides (AHA, 2000)¹⁶. Furthermore, omega-3 fatty acids in the diet confer additional cardioprotective effects beyond their consistent, significant improvement in blood lipid profile by reducing triglycerides. Reviews of metabolic, epidemiological and large scale dietary studies in normal humans have shown little or no incremental coronary heart disease risk associated with consumption of one egg or about 200 mg cholesterol/day from eggs. On the basis of the above, FDA should take into account these most recent findings and conclusions regarding type of fat intake and the overall positive health benefits from the nutritive values in omega-3 enhanced eggs. Therefore, we request that FDA grant an exception to the disqualifying cholesterol limit for this qualified health claim for consumption of up to one Columbus® or Christopher® egg/day.

6) 21 CFR §101.14(e)(6) Nutrient contribution to the diet: Omega-3 enhanced eggs meet the requirement

A standard egg as well as the omega-3 fatty acid enriched egg that is the subject of this petition provide 10 percent of the daily reference value for protein in the diet and therefore, meet the criteria for exemption from prohibition. (www.aeb.org/food/nutrition.html)

B) SUMMARY OF SCIENTIFIC DATA

1) Historical Perspective on Modern Dietary Patterns

Several articles have commented on the changes in dietary patterns of the modern Western diet in comparison to the diet of ancestral hunter-gatherers and possible implications for chronic disease ((Eaton *et al.*, 1988)¹⁷, (Simoupolos, 1998 and 2002)^{18,19}. As shown in Table 1, there are a number of significant differences between the wild-type diet and modern diet regarding energy and fat intake. In particular, there are notable shifts in the type and amount of fats, oils and essential fatty acid content of food.

Table 1 Dietary Changes Associated with Modern Diets vs. Early Human Diets

Characteristic	Hunter-gatherer diet and lifestyle	Western diet and lifestyle
Energy density	Low	High
Energy intake	Moderate	High
Protein	High	Low-moderate
Animal	High	Low-moderate
Vegetable	Very low	Low-moderate
Carbohydrate	Low-moderate (slowly absorbed)	Moderate (rapidly absorbed)
Fiber	High	Low
Fat	Low	High
Animal	Low	High
Vegetable	Very low	Moderate to high
Total long-chain ω 6 + ω 3	High (2.3 g/d)	Low (0.2 g/d)

Modified from Simoupolos, 2002

This change in diet has become most pronounced in the last 150 years with the advent of industrialized agriculture (Figure 1). The hunter-gatherer ate a variety of wild plants and game animals; modern diets rely upon three cereal grains rich in omega-6 fatty acids, wheat, corn and rice, for the majority of the food supply. And yet, for most of man's existence, humans rarely or never consumed these types of cereal grains. In addition, the technology for large scale production of vegetable oil from soybeans, corn and other oilseeds and their hydrogenated derivatives resulted in lowered omega-3 content and formation of saturated fats and *trans*-fatty acids. Refined sugars, a dense source of energy, have shown an enormous increase in consumption over the last 40 years, largely as a result of intake of high-fructose corn syrup in soft drinks (Putnam *et al.*, 2002). Lastly, the modern practices of feeding livestock with grains containing high

amounts of omega-6 fatty acids, but largely deficient in omega-3 fatty acids, have resulted in fatty meats with relatively little omega-3 fatty acid content compared to lean, wild game. In summary, the diets of industrialized societies are characterized by (1) an increase in energy intake (fats, sugars) and decrease in energy expenditure (exercise); (2) an increase in saturated fat, omega-6 fatty acids and *trans*-fatty acids, and a decrease in omega-3 fatty acid intake; (3) a decrease in complex carbohydrates and fiber; and (4) an increase in cereal grains and a decrease in fruits and vegetables.

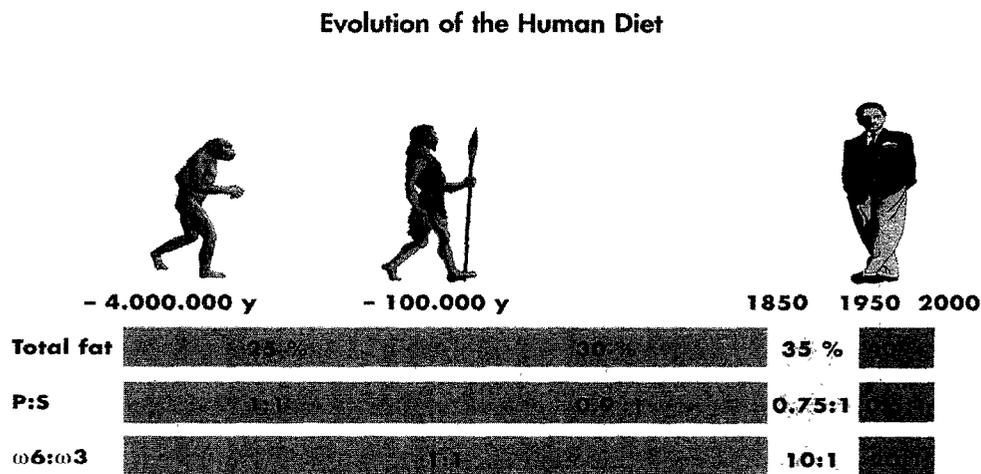


Figure 1. Historical timeline of human dietary fat and fatty acid intakes

As will be demonstrated in later sections of the petition, the balance or ratio of omega-6 to omega-3 intake is critically important in the pathogenesis of chronic diseases such as cardiovascular disease. In hunter-gatherer societies, this ratio was approximately 0.79 in comparison to the current ratio in the U.S diet of 16.74 (Eaton *et al.*, 1998)²⁰. The Mediterranean diet prior to 1960 had an omega-6 to omega-3 ratio of 1-2, current northern Europe, a ratio of 15 (Sanders, 2000)²¹ and the current Japanese diet has an omega-6 to omega-3 ratio of 4 (Sugano and Hirahara, 2000)²². Bang and Dyerberg (1980)²³ conducted a seminal study in Greenland Eskimos where they found the ratio of omega-6 to omega-3 is approximately 1 in platelet phospholipids, a marker for omega 6/omega 3 intake from fish. There appears to be a correlation between lowered omega-6 to omega-3 ratios and the rates of cardiovascular disease mortality. In the US and

northern European, 45% of all deaths are from cardiovascular disease, in Japan, 12% and in Greenland Eskimos, 7%.

2) Essential Fatty Acids and Metabolism

Fatty acids are long linear hydrocarbon chains containing from four to 30 hydrocarbons, most commonly 12 to 24 carbons. One end of the molecule contains a carboxylic acid group from which chemists count the number of carbons. The other end is the methyl, "n" or omega end from which nutritionists and biochemists count the position of the first double bond. Location of the first double bond end determines whether the fatty acid is an omega-6 or omega-3 fatty acid. Nutritionists use shorthand to describe fatty acids by their chain length, number of double bonds and location of the first carbon with a double bond (www.pufanewsletter.com).

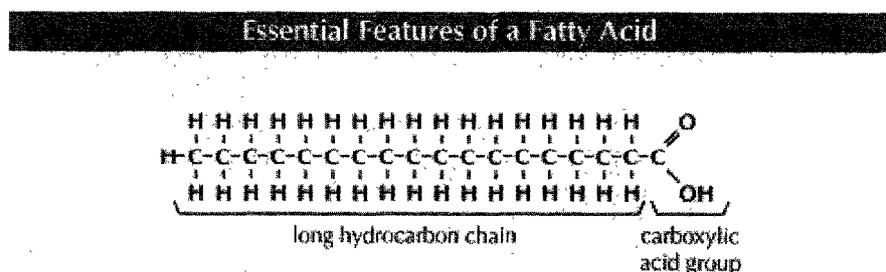
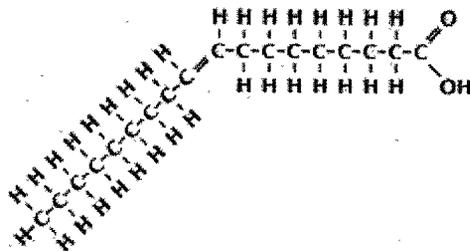


Figure 2. Saturated fatty acid

Fatty acids having their full complement of hydrogen atoms are termed saturated (Figure 2). These exist as straight chains. The most abundant saturated fatty acid in nature is palmitic acid, a 16-carbon fatty acid (16:0). If two hydrogen atoms are removed from the chain, a carbon-to-carbon double bond or point of unsaturation is created and the molecule bends.

Carbon-to-Carbon Double Bond



The above diagram depicts Oleic acid, a monounsaturated fatty acid. Note that the double bond is *cis*; this is the common natural configuration.

Figure 3. Monounsaturated fatty acid

This bending causes the molecule to occupy more space and become more fluid. Fatty acids with one double bond are called monounsaturated (Figure 3). The most common monounsaturated fatty acid is oleic acid, an 18-carbon fatty acid with its double bond nine carbons from the methyl end (18:1 ω 9). Oleic acid is the major fatty acid in olive oil. Polyunsaturated fatty acids (PUFA) have two or more double bonds and are most common in the omega-6 and omega-3 structures. For example, linoleic acid (LA) is an 18-carbon omega-6 fatty acid (18:2 ω 6), with two double bonds. LA is by far the most abundant polyunsaturated fatty acid in most human tissues. It is abundant in plants, cereals and seed oils. Its counterpart in the omega-3 class is alpha-linolenic acid (ALA), an 18-carbon fatty acid with three double bonds (18:3 ω 3). Many plants produce this polyunsaturated fatty acid, but because of the small amounts of fresh vegetables consumed, it is one of the least abundant of the essential fatty acids in most diets. It is found in at relatively high levels in flax, hemp, rape (canola) seed, soybean, and in walnut, chia and perilla oils.

Table 3. Essential Fatty Acid Nomenclature

<i>Omega</i> ω 3 group	Acronym	Carbon:#: # double bonds/ 1 st carbon position from methyl group
<i>alpha</i> -Linolenic acid	ALA	18:3 ω 3
Eicosapentaenoic acid	EPA	20:5 ω 3
Docosapentaenoic acid	DPA	22:5 ω 3
Docosahexaenoic acid	DHA	22:6 ω 3
<i>Omega</i> ω 6 group		
Linoleic acid	LA	18:2 ω 6
<i>gamma</i> -linolenic acid	GLA	18:3 ω 6
Arachidonic acid	ARA	20:4 ω 6
Docosatetraenoic acid	DTA	22:4 ω 6

Humans are able to synthesize from carbohydrates and protein the saturated and monounsaturated fatty acids necessary for normal cellular and neurological functions. The essential fatty acids (EFA), by definition, cannot be synthesized and must be obtained from the diet. The reason that mammals require EFA such as linoleic acid (LA) and alpha-linolenic acid (ALA) is that they lack the two enzymes ($\Delta 5$ & $\Delta 6$ desaturases) that can desaturate the carbon chain downstream from the methyl end. EFA metabolism occurs by a series of desaturation and chain elongation steps, which originate at the carboxyl terminus. As shown in the Figure 4 below, the short chain ALA is metabolized from ALA to EPA to DPA and the 22 carbon DHA through desaturation and chain elongation into a long chain PUFA. Likewise, LA is desaturated to four double bonds and elongated to 20 carbons to form a long chain PUFA, usually arachidonic acid (AA). However, in neither metabolic series is an omega-3 fatty acid or an omega-6 fatty acid able to be interconverted to an omega-6 or omega-3 fatty acid; the first double bond always remains in its original position.

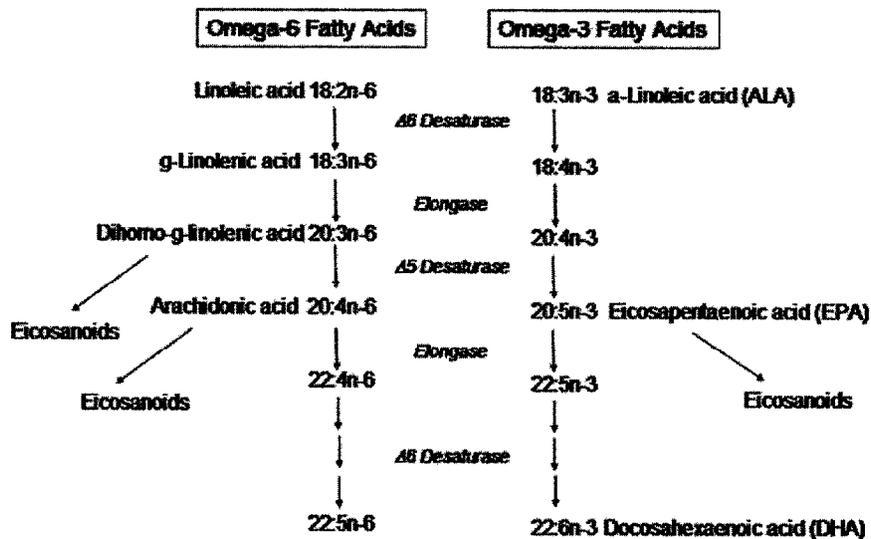


Figure 4. PUFA Desaturation and Elongation

One of the key functions of the essential fatty acids is to supply the precursor compounds for eicosanoid formation. Long chain PUFA such as AA and EPA are stored as membrane phospholipids and are present in triglycerides in adipose tissue. AA is the major precursor for the synthesis of eicosanoids, powerful cellular regulatory substances and mediators of inflammation that include prostaglandins, thromboxanes, and leukotrienes. Eicosanoids are involved in platelet aggregation, T-cell proliferation, lymphocyte migration, vasoconstriction and dilation, and the production of several immune and inflammatory substances. In the presence of EPA, eicosanoids derived from the omega-3 family will also be produced, and these attenuate or inhibit the action of the AA-derived eicosanoids. Those derived from EPA also have weaker biological activity than those from AA. Competition from omega-3 fatty acids for the same eicosanoid synthesizing enzymes reduces the production of omega-6 eicosanoids. Thus, the presence of omega-3 fatty acids such as EPA can moderate the production and activity of AA-derived eicosanoids.

3) Role of Omega-3 and Omega-6 Fatty Acids in Eicosanoid Formation and Their Physiological Effects

The eicosanoids consist of the prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs). All mammalian cells except erythrocytes synthesize eicosanoids. These molecules are extremely potent with hormone-like effects and are able to cause profound physiological effects at very dilute concentrations. All eicosanoids function locally at the site of synthesis, through receptor-mediated G-protein linked signaling pathways leading to an increase in intracellular cAMP levels. The eicosanoids produce a wide range of biological effects on inflammatory responses, on the intensity and duration of pain, fever, and immune function. They also play important roles in inhibiting gastric acid secretion, regulating blood pressure through vasodilation or constriction, and inhibiting or activating platelet aggregation and thrombosis.

The principal eicosanoids of biological significance to humans are a group of molecules derived primarily from arachidonic acid (AA), an omega-6 fatty acid, and secondarily, from eicosapentaenoic acid (EPA), an omega-3 fatty acid. Within the cell,

AA and EPA reside predominantly in the cell membrane lipid bilayer as membrane phospholipids and are released upon the activation of phospholipase A₂

Three main pathways are involved in the biosynthesis of eicosanoids. The prostaglandins and thromboxanes are synthesized by the cyclic pathway, the leukotrienes, such as hydroxyl acids and lipoxins are synthesized by the linear pathway. A lesser pathway is the epoxygenase pathway, which introduces oxygen into the double bonds by P-450 epoxygenases to form epoxy acids. Numerous stimuli such as epinephrine, bradykinin or thrombin activate phospholipase A₂ that hydrolyzes AA and EPA from membrane phospholipids. The cyclic pathway is initiated through the action of prostaglandin G/H synthase (PGS). This enzyme possesses two activities, cyclooxygenase (COX) and peroxidase. There are two forms of the COX activity. COX-1 (PGS-1) is expressed constitutively in gastric mucosa, kidney, platelets and vascular endothelial cells. COX-2 (PGS-2) is inducible and is expressed in macrophages and monocytes in response to inflammation. The primary trigger for COX-2 induction in monocytes and macrophages is platelet-activating factor, PAF and interleukin 1. COX-2 catalyzes the two-step conversion of AA and EPA to PGG₂ and then to PGH₂. The linear pathway is initiated through the action of lipoxygenases. It is the enzyme, 5-lipoxygenase that gives rise to the leukotrienes. Table 4 below summarizes the sources and biological activities of the various prostaglandins, leukotrienes and thromboxanes.

A widely used class of drugs, the non-steroidal anti-inflammatory drugs (NSAIDs) including ibuprofen, indomethacin, naproxen, phenylbutazone and aspirin, all act upon the cyclooxygenase activity, inhibiting both COX-1 and COX-2. Because inhibition of COX-1 activity in the gut is associated with NSAID-induced ulcerations, pharmaceutical companies have developed drugs targeted exclusively against the inducible COX-2 activity (e.g. celecoxib and rofecoxib). Another class, the corticosteroid drugs, acts to inhibit phospholipase A₂, thereby inhibiting the release of fatty acids from membrane phospholipids and the subsequent synthesis of eicosanoids.

Table 4. Biological Activity of Major Eicosanoid Mediators

Eicosanoid	Major sites of synthesis	Major biological activities
PGD ₂	mast cells	inhibits platelet and leukocyte aggregation, decreases T-cell proliferation and lymphocyte migration and secretion of IL-1 and IL-2; induces vasodilation and production of cAMP
PGE ₂	kidney, spleen, heart	increases vasodilation and cAMP production, enhancement of the effects of bradykinin and histamine, induction of uterine contractions and of platelet aggregation, maintaining the open passageway of the fetal ductus arteriosus; decreases T-cell proliferation and lymphocyte migration and secretion of IL-1 and IL-2
PGF _{2α}	kidney, spleen, heart	increases vasoconstriction, bronchoconstriction and smooth muscle contraction
PGH ₂		precursor to thromboxanes A ₂ and B ₂ , induction of platelet aggregation and vasoconstriction
PGI ₂	heart, vascular endothelial cells	inhibits platelet and leukocyte aggregation, decreases T-cell proliferation and lymphocyte migration and secretion of IL-1 and IL-2; induces vasodilation and production of cAMP
TXA ₂	platelets	induces platelet aggregation, vasoconstriction, lymphocyte proliferation and bronchoconstriction
TXB ₂	platelets	induces vasoconstriction
LTB ₄	monocytes, basophils, neutrophils, eosinophils, mast cells, epithelial cells	induces leukocyte chemotaxis and aggregation, vascular permeability, T-cell proliferation and secretion of INF-γ, IL-1 and IL-2
LTC ₄	monocytes and alveolar macrophages, basophils, eosinophils, mast cells, epithelial cells	component of SRS-A, microvascular vasoconstrictor, vascular permeability and bronchoconstriction and secretion of INF-γ
LTD ₄	monocytes and alveolar macrophages, eosinophils, mast cells, epithelial cells	microvascular vasoconstrictor, vascular permeability and bronchoconstriction and secretion of INF-γ
LTE ₄	mast cells and basophils	microvascular vasoconstrictor and bronchoconstriction

As the above table demonstrates, the eicosanoids are intimately involved in the regulation of blood viscosity, platelet and leukocyte aggregation, vasoconstriction, inflammation and immune cellular response. Because the PUFA composition of cell membranes, particularly platelets, neutrophils, monocytes and liver cells, is largely dependent on dietary intakes, an excessive intake of omega-6 PUFA such as arachidonic acid can result in excessive production of the potent eicosanoids. PGS-1 and PGS-2 have a much greater specificity and are able to better utilize arachidonic acid than eicosapentaenoic acid. Further, DHA is a competitive inhibitor of PS-1 and PS-2 and does not form eicosanoid products. Therefore, the presence of omega-3 fatty acids both competes for enzyme binding and inhibits eicosanoid formation from AA, thereby modulating and reducing the prostaglandin, thromboxane and leukotriene responses to

stimuli. Furthermore, the eicosanoid products from EPA metabolism are generally less potent and bind less selectively to prostanoid receptors (Abramovitz et al., 2000)²⁴. Thus, a diet with a high omega-6/omega-3 PUFA ratio will have a higher eicosanoid production which shifts the hematological system to a prothrombic, proaggregatory state and a vascular system to a more vasoconstrictive status (Cartwright et al., 1985)²⁵.

Simonopoulos (2002)¹⁹ has reviewed the experimental studies on the effect of omega-3 fatty acids on inflammatory reactions and a summary of the effects of omega-3 fatty acids is presented in Table 5 below. Omega-3 fatty acids are considered to have numerous mitigating effects on the factors that tend to enhance the inflammatory response. Inflammation is an important factor in the development of atherosclerosis and thrombosis commonly observed in patients with cardiovascular disease.

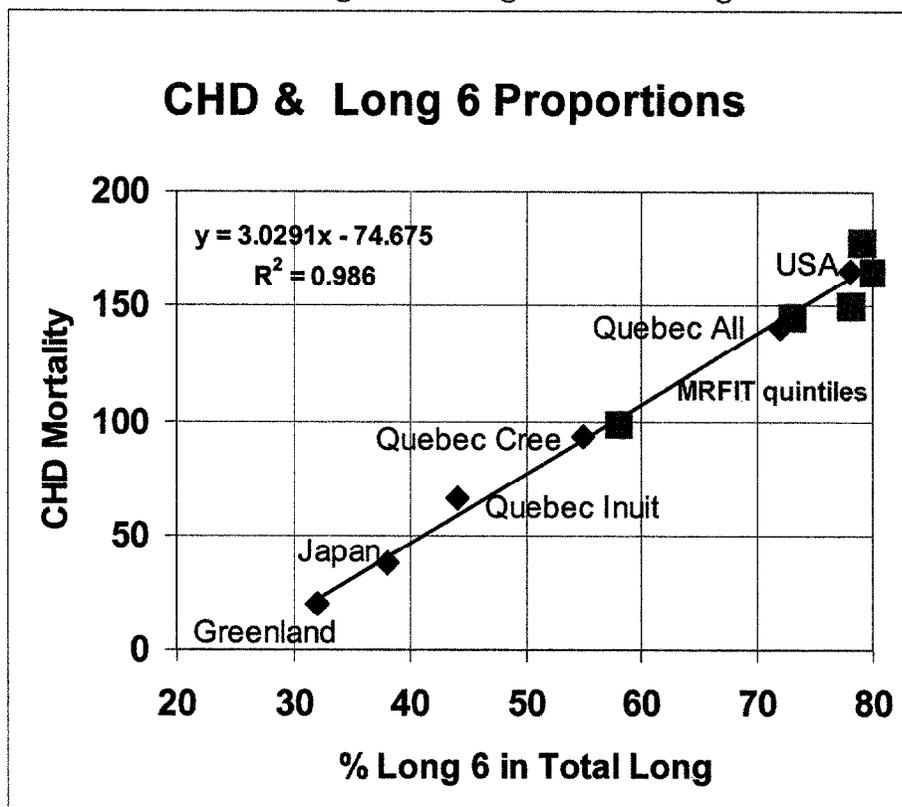
Table 5 Effect of omega-3 fatty acids on factors involved in the pathophysiology of inflammation

Factor	Function	Effect of ω 3 fatty acids
Arachidonic acid	Eicosanoid precursor; aggregates platelets; stimulates white blood cells	↓↓↓
Thromboxane	Platelet aggregation; vasoconstriction; increase of intracellular Ca ⁺	↓↓↓
Prostacyclin (PGI _{2/3})	Prevent platelet aggregation; vasodilation; increase camp	↑↑↑
Leukotriene (LTB ₄)	Neutrophil chemoattractant; increase of intracellular Ca ⁺	↓↓↓
Fibrinogen	A member of the acute phase response; and a blood clotting factor	↓↓↓
Tissue plasminogen activator	Increase endogenous fibrinolysis	↑↑↑
Lipid hydroperoxides	Stimulate eicosanoid formation	↓↓↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil O ₂ free radical formation; stimulate lymphocyte proliferation; stimulate PAF; express intercellular adhesion molecule-1 on endothelial cells; inhibit plasminogen activator and procoagulants	↓↓↓
Platelet activating factor (PAF)	Activates platelets and white blood cells	↓↓↓
PDGF	Chemoattractant and mitogen for smooth muscles and macrophages	↓↓↓
Oxygen free radicals	Cellular damage; enhance LDL uptake via scavenger pathway; stimulate arachidonic acid metabolism	↓↓↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil O ₂ free radical formation; stimulate lymphocyte proliferation; stimulate PAF; express intercellular adhesion molecule-1 on endothelial cells; inhibit plasminogen activator, thus, procoagulants	↓↓↓
Interleukin-6	Stimulates the synthesis of all acute phase proteins involved in the inflammatory response: C-reactive protein; serum amyloid A; fibrinogen; α 1-chymotrypsin; and haptoglobin	↓↓↓

The above review of omega-3 and omega-6 fatty acid metabolism to eicosanoids and their modulating effect on various factors involved in inflammatory response, thrombosis and atherosclerosis formation, immune function and coagulation is not comprehensive, but is meant to be illustrative of the multiple, diverse actions of omega-3

and omega-6 fatty acids on cellular, hematological and vascular responses. The proportion of the diet that is omega-3 versus omega-6 fatty acids is reflected in the relative proportions found as tissue long chain omega-3 or omega-6. The more omega-6 and less omega-3 fatty acids present in the tissues for eicosanoid formation, the more formation and intense action of the omega-6 eicosanoids and likely resulting pathological effects over the lifespan of the individual. On a population basis, Figure 5 clearly shows the relationship between the proportions of long chain fatty acids of omega-6 or omega-3 origin in the body tissues and mortality from coronary heart disease (efaeducation.nih.gov/sig/personal1.html). The coronary heart disease axis represents age-adjusted annual mortality rates per 100,000. As the percent of long chain omega-6 fatty acids increases from 20% to 80% of total long chain PUFA, mortality increases from 20 to 200 deaths per 100,000.

Figure 5. Age adjusted mortality/100,000 from CHD vs. % long chain omega-6 of total long chain PUFA



In summary, even though chronic diseases such as cardiovascular disease are multifactorial in causation, there is a substantial body of scientific evidence that establishes the biological plausibility and mechanisms for the prevention or mitigation of the pathophysiological conditions leading to cardiovascular disease from a balanced intake of omega-3/omega-6 fatty acids.

4) Observational Studies on Populations

The relationship between the intake of omega-3 fatty acids and reductions in the rates of cardiovascular disease and related mortality was first identified by studies conducted in the 1970's and early 1980's on human subpopulations known to have remarkably low incidences of these diseases. These subpopulations included Alaskan Native Americans, Greenland Eskimos, and Japanese fishing villagers. Common to all of these groups was a high intake of fish and other marine animals, which suggested that fish oils may confer protection against atherosclerosis. These studies are summarized in Table 6.

The pioneering findings of Bang and Dyerberg (1980)²³ regarding the relationship of fish consumption and nutritional lipid patterns to cardiovascular disease in Greenland Eskimos are seminal in understanding the mechanisms by which the omega-3/omega-6 fatty acid ratio in the diet is cardioprotective. Despite the fact that their diet was composed of 39% fat and mid-range intakes up to 304 g fat/day, Greenland Eskimos had lower levels of plasma cholesterol and LDL cholesterol and higher levels of HDL cholesterol compared to Danes consuming 42% of their diet as fat. The most remarkable finding was the shift in omega-3 and omega-6 fatty acid intakes between the two societies. Whereas the Eskimos had a daily intake of 5.4g of omega-6 fatty acids and 13.7 grams omega-3 fatty acids /3000 kcal intake (ratio 0.39), Danes had an intake of 10 g omega-6 and 2.8 g omega-3 fatty acids (ratio 3.6), which is still low compared to modern Western diets with ratios of 15-20. The dietary pattern was reflected in the fatty acid composition in platelets with an omega-3/omega-6 ratio of 0.94 in Eskimos and 0.02 in Danes. This led these authors to conclude that the most important factor in reducing ischemic heart disease and other thrombotic diseases was not the changes in blood lipid

profile, but the reduction in thrombosis and atherosclerosis due to the ratio shifts in structural lipids and changes in prostaglandin balance toward an antiaggregatory state. This is likely the cause of the low incidence of myocardial infarction seen by Kroman *et al.* (1980)²⁶ in the Greenland Eskimo population

These findings were supported by the studies conducted on Japanese fishing villagers and farmers. (Hirai *et al.*, 1989)²⁷. Residents of the fishing village who consumed 5.4 g/day of omega-3 fatty acids (EPA and DHA) had significantly lower mortality than farmers (1.5 g/day omega-3/day) from ischemic heart disease and cerebrovascular events. As was seen in the Eskimos, platelet aggregability was notably reduced in the fishing villagers. Residents of Okinawa with high fish intakes also had rates of coronary heart and cerebrovascular disease that was 50% of mainland Japan residents (Kagawa *et al.* (1982)²⁸. In summary, all of the observational studies in populations with diets high in omega-3 intake and relatively low omega-6 intake have low rates of cardiovascular disease mortality compared to groups with lower omega-3 intakes.

Table 6. Observational studies of subpopulations with high dietary fish intake

Study reference	Study type	No. of subjects	Outcome
Bang and Dyerberg, 1980	Observational	NS	Ischemic heart disease accounted for only 3.5% of all deaths in Greenland; Greenland Eskimo diet has 5.4 g/day omega-6 fatty acids and 13.7 g/d omega-3 fatty acids (ratio 0.39); EPA, DPA and DHA constitute 13% of fatty acids vs. 0.8% in Danish food.
Hirai <i>et al.</i> , 1980 ²⁹	Observational	NS	Significant reduction in ischemic heart and cerebrovascular mortality in Japanese fishing villagers vs. farmers associated with higher fish intake; also reduced platelet aggregation
Kroman <i>et al.</i> , 1980	Observational	1887	Low incidence of myocardial infarction (3 actual vs. 40 expected) in a Greenland whaling/fishing community
Kagawa <i>et al.</i> , 1982	Observational	66	Approximately 50% decrease in ischemic heart and cerebrovascular disease in Okinawa vs. mainland Japan associated with a significant increase in serum EPA levels
Middaugh, 1990 ³⁰	Observational	NS	Annual, age adjusted death rates in Alaska Natives markedly lower than rates in non-natives (162 vs. 242; RR 0.67)
Newman <i>et al.</i> , 1993 ³¹	Observational (Autopsy examination of atherosclerosis)	103 Natives 101 non-Natives	All subjects died of accidental, non-cardiovascular, causes. Native Alaskans had significantly less atherosclerosis as measured by mean percent of vessel surface with atherosclerotic lesions, fatty streaks and raised lesions than non-Natives.
Rodriguez <i>et al.</i> , 1996 ³²	Observational	8006	High fish intake reduced the risk of CHD incidence and mortality by 50% in heavy smokers

5) Prospective Cohort Studies

A summary of published articles on the relationship of cardiovascular disease to omega-3 fatty acid intake in the diet from fish and other sources is given in Table 7. The great majority of these studies have demonstrated significantly reduced risks of cardiovascular disease and death associated with increased intake of omega-3 fatty acids. Of the two studies that showed little or no cardioprotective effects of fish intake, the finding of Morris *et al.* (1995)³³ in the Physicians Health Study that there was no effect on cardiovascular risk from fish intake at 4 years follow-up were not confirmed by Albert *et al.* (1998)³⁴ who found a 52% reduction in sudden cardiac death associated with 1 fish meal per week after 11 years follow-up of the same group of physicians. The lack of any

effect on ischemic heart disease risk in a health-conscious population including vegetarians in the UK with a 50% lower risk of heart disease than the general UK population is not surprising considering the increased amounts of omega-3 fatty acids that would likely be consumed in replacement foods such as nuts and greens and the concomitant reduction in omega-6 fatty acid intake (Mann *et al.*, 1997)³⁵.

Krumhout *et al.* (1985, 1995 and 1998)^{36 37 38} were the first investigators to show reductions in coronary heart disease mortality (approximately 50%) associated with fish intake (24-30 g/day) in a Dutch population. This finding was confirmed in the Western Electric study by Daviglius *et al.* (1997)³⁹ who reported a 40% reduction in fatal coronary heart disease in men who consumed 35 g or more of fish daily. Even in populations with one or more risk factors such as smoking or fatty diets (Dolacek, 1992⁴⁰; Pietinen *et al.*, 1997)⁴¹, coronary disease mortality was significantly reduced (25-40%) in association with increased ALA intake from fish. Iso *et al.* (2001)⁴² have reported significant dose-related relative risk reductions of stroke in women who consume fish once per week, 0.78, 2 times a week, 0.73 and 5 times a week, 0.48.

Two studies recently conducted by Hu and his colleagues offer the strongest evidence to date on the beneficial effects of fish, omega-3 and specifically ALA intake and reduction of coronary heart disease. Using dietary questionnaires from over 76,000 nurses, the amount of ALA in the diet was calculated and compared to cardiovascular risks (Hu *et al.*, 1999 and 2002)^{43 44}. Women were divided into quintiles based on consumption of ALA (0.71, 0.86, 0.98, 1.12 and 1.36 g/day); the relative risk of fatal ischemic heart disease from the lowest to highest intake were 1.0, 0.99, 0.90, 0.67, and 0.55. The trend analysis was significant for an inverse association between ALA intake and fatal coronary disease. Nonfatal myocardial infarction was only slightly decreased (RR= 0.85) in comparing the lowest to highest intakes of ALA. In another analysis of the Nurses Health Study, fish and omega-3 consumption was evaluated against the risks of coronary heart disease (Hu *et al.*, 2002)⁴⁴. The relative risks for coronary heart disease for those consuming fish <1/month, 1-3/month, 1/week, 2-4 times/week and >5 times/week were 1.0, 0.79, 0.64, 0.71, 0.69 and 0.66, respectively. Further, there were significant

trends for risk reduction of fatal coronary heart disease, total coronary heart disease and nonfatal myocardial infarction in association with increasing consumption of omega-3 fatty acids. No notable differences in risk reduction were found in groups with high (9.60 versus low (5.9) ratios of omega-6 to omega-3 intake. The risk reduction of coronary heart disease death (RR=0.55 for lowest intake versus highest intake of omega-3) was greater than for nonfatal myocardial infarction (RR=0.73). These investigators considered the beneficial effects of omega-3 fatty acids and ALA to be the result of their antiarrhythmic and antithrombotic actions.

In summary, the published prospective cohort studies provide a consistent and convincing body of evidence that increased intake of omega-3 fatty acids from fish, oils, nuts and other dietary sources can significantly decrease the risk of sudden cardiac death, total mortality from coronary heart disease and coronary heart disease events including nonfatal myocardial infarction. One study has shown significant reduction in stroke risk associated with fish intake. The findings of these studies are applicable to the general U.S. population in their implications for reduction of cardiovascular disease.

Table 7. Prospective cohort studies investigating the role of diet and cardiovascular disease risk

Study reference	Study type		No. of subjects	Duration/ Follow-up (years)	Age range	Outcome
Krumhout <i>et al.</i> , 1985	Prospective cohort study of normal male population in the Netherlands:	Dietary questionnaires on fish intake	852	20	40-59	Mortality from CHD decreased 50% in men consuming 30 g of fish/d
Dolacek., 1992	Prospective cohort study of multiple risk factor group of US men (smoking, high BP, and serum cholesterol):	Dietary questionnaire to assess total PUFA intake by quintiles	6250	10.5	35-57	No effects on any cause of mortality associated with omega-6 PUFA intake; significant inverse association for mortality from CHD, CVD and all causes were associated with ALA and omega-3 PUFA intake
Krumhout <i>et al.</i> , 1995	Prospective cohort study of elderly Dutch	Dietary questionnaires on fish intake	272	17		Relative risk of CHD death 0.51 with intake of 24 g/d fish compared to no intake

Table 7. Prospective cohort studies investigating the role of diet and cardiovascular disease risk

Study reference	Study type		No. of subjects	Duration/ Follow-up (years)	Age range	Outcome
Morris <i>et al.</i> , 1995	population Prospective cohort study of healthy US male physician group	Dietary questionnaire	21,185	4	40-84	Fish consumption not associated with reduced risk of myocardial infarction, cardiovascular event or cardiovascular mortality
Ascherio <i>et al.</i> , 1996 ⁴⁵	Prospective cohort study of healthy US male physician group	Dietary questionnaire	44,757	6	40-75	Multivariate risk reduction in MI of 0.41 and fatal CHD of 0.57 with 1% increase in energy from ALA intake; non-significant inverse relationship of ALA and CHD
Mann <i>et al.</i> , 1997	Prospective cohort study of health conscious persons in the UK including vegetarians	Dietary questionnaires	10,802	13.3	16-79	Death rate for ischemic heart disease 50% of all persons in UK; no additional cardioprotective effect for individuals eating fish more than once/week
Daviglus <i>et al.</i> , 1997.	Prospective cohort study of healthy US male group	Dietary questionnaires	1822	30	40-55	Relative risk of CHD and sudden or non-sudden death from MI 0.62 for men consuming 35 g/day fish and RR for non-sudden MI 0.33; significant inverse trend for CHD with increasing fish intake
Pietinen <i>et al.</i> , 1997	Prospective cohort study of CVD-free Finnish male smokers with high fat diets	Baseline dietary questionnaires	21,930	6.1	50-69	Multivariate relative risk of coronary death of 1.30 for highest quintile intake of omega-3 fatty acids; highest ALA intake had a risk ratio of 0.75.
Albert <i>et al.</i> , 1998	Prospective cohort study of normal male physician population	Dietary questionnaires on fish intake	20,551	11	40-84	Multivariate relative risk of sudden cardiac death 0.48 associated with 1 fish meal/week; Fish or omega-3 intake not associated with reduced risk of total MI, nonsudden cardiac death,

Table 7. Prospective cohort studies investigating the role of diet and cardiovascular disease risk

Study reference	Study type		No. of subjects	Duration/ Follow-up (years)	Age range	Outcome
Hu <i>et al.</i> , 1999	Prospective cohort study of healthy population of US nurses	Estimated intake of ALA and other nutrients from dietary questionnaire	76,283	10	30-55	or total CVD mortality Multivariate relative risk for women consuming 1.2 or 1.36 g/d ALA for fatal CHD, 0.67 and 0.55; nonfatal MI, 1.02 and 0.85; respectively
Oomen <i>et al.</i> , 2000 (Seven Country) ⁴⁶	Prospective cohort study in normal male populations of Italy, Netherlands and Finland:	1-19, 20-39 or >40 g/day fish; mean intake; 20, 18 and 39 g/d fish respectively,	833 396 612	20	40-59	No notable change in relative risk (pooled 1.08 RR for highest quartile vs. no intake) in CHD mortality associated with total fish intake; pooled relative risk for CHD associated with fatty fish intake, 0.66.
Rissanen <i>et al.</i> , 2000 ⁴⁷	Prospective cohort study of healthy Finnish men	Dietary questionnaire	1871	10	42-60	Relative risk of acute coronary events reduced 44% in quartile with highest DHA and DPA serum levels; no association with EPA levels
Iso <i>et al.</i> , 2001	Prospective cohort study of healthy population of US nurses:	Intake of omega-3 PUFA from dietary questionnaire	79,839 women	14	34-59	Multivariate relative risk of stroke from consuming 1 serving/wk, 0.78; 2-4x/wk, 0.73 and 5x/wk, 0.48. Relative risk of thrombotic infarction for 2x/wk, 0.49. No association with fish intake and hemorrhagic stroke found.
Albert <i>et al.</i> , 2002 ⁴⁸	Prospective cohort study of normal male physician population:	Dietary questionnaires on nut intake	21,454 males	17	NS	Multivariate relative risk for men consuming >2 servings/wk of nuts for sudden cardiac death, 0.53 and CHD, 0.70. No reduced risk of non-fatal MI or non-sudden CHD deaths.

Table 7. Prospective cohort studies investigating the role of diet and cardiovascular disease risk

Study reference	Study type		No. of subjects	Duration/ Follow-up (years)	Age range	Outcome
Hu <i>et al.</i> , 2002	Prospective cohort study of healthy population of US nurses	Estimated fish intake from dietary questionnaire	76,283	10	30-55	Significant inverse association of fish consumption and total CHD, fatal CHD and non-fatal MI; multivariate relative risk of 5x/week fish meals vs. <1/mo is 0.60 for total CHD, 0.55 for fatal CHD and 0.77 for nonfatal MI; multivariate risk of 1/week fish meal 0.79 for all three measures

6) Case Control Studies

Recent case control studies that have evaluated the association between moderate intake of omega-3 fatty acids, membrane fatty acid composition and risks of myocardial infarction and/or sudden death. The findings of these studies are summarized in Table 8. Tavani *et al.* (2001)⁴⁹ compared the omega-3 intakes from fish meals of patients with nonfatal acute myocardial infarctions (AMI) with hospital controls without cardiovascular disease in low risk (nonsmokers, low total cholesterol) and high risk (smokers, high cholesterol, and family history of MI) groups. Fish intake was estimated by dietary questionnaire into groups with less than 1 fish meal/week, 1-2 fish meals/week and greater than 2 fish meals/week. The estimated intakes of omega-3 PUFA at these fish meal cutoff points were 0.81 and 1.28 g/omega-3/week. Multivariate analysis showed a significant inverse trend between nonfatal AMI and fish consumption, with an overall odds ratio of 0.67 for AMI for intermediate and high consumption. High risk patients had a stronger inverse association of AMI risk and fish intake, but moderate fish consumption also reduced AMI risk in the low risk population.

Studies in animals and myocardial cell cultures have shown that omega-3 fatty acids have antiarrhythmic effects, possibly through an effect on membrane fatty acid composition and modulation of sodium and calcium channels (Kang and Leaf, 2000)⁵⁰.

There is evidence that omega-3 PUFA is cardioprotective against ischemia-induced ventricular fibrillation and sudden death in animal studies (McLennan, 1993⁵¹; Billman *et al.*, 1999⁵²) and in human studies where increased omega-3 intake was associated with reduced risk of sudden cardiac death (Albert *et al.*, 1998; Hu *et al.*, 1999)^{34 43}. In order to investigate this hypothesis, two case-control studies have evaluated the risks of sudden death or primary cardiac arrest and their association with dietary intake of omega-3 fatty acids and by a biomarker of membrane incorporation, namely percentage of long-chain omega-3 fatty acids of total red blood cell membrane fatty acids or blood fatty acid composition. Siscovick *et al.* (2000)⁵³ found that a modest intake of 5.5 g/month of omega-3 fatty acids or about 1 fatty fish meal/week conferred a 50% reduction in risk of primary cardiac arrest; higher intakes of fish were reported to have little additional risk reduction. Further, there was a significant inverse association between RBC membrane omega-3 percentage and primary cardiac arrest. These findings were considered consistent with the dietary intake-induced changes in long chain omega-3 fatty acid composition of cell membranes and reduced susceptibility to life-threatening cardiac arrhythmias.

Albert *et al.* (2002)⁵⁴ have confirmed these findings in a study of previously healthy patients who had suffered sudden cardiac deaths likely due to cardiac arrhythmia compared to two healthy matched controls. Baseline blood samples were analyzed for saturated, monosaturated and polyunsaturated fatty acids, including omega-6 and omega-3 fatty acids separately. Control and sudden death subjects were segregated by quartiles of percent omega-3 fatty acid in the blood as follows: 3.58%, 4.76%, 5.63% and 6.87%. There was a significant, inverse and linear trend in the association of percent omega-3 fatty acids in blood and risk of sudden death. In comparison to the lowest quartile, there were 45, 72 and 81% reductions in sudden cardiac death risk for increasing percentage quartiles of omega-3 fatty acids. In contrast to the prior study where there was a reported threshold of fish intake benefit, no threshold for blood levels on risk reduction was noted.

In summary, the case control studies provide substantial evidence that moderate omega-3 intake, as reflected in the diet and particularly as a percentage of the cell

membrane fatty acids, results in remarkable reduction in risk from sudden cardiac death. The presence of increasing amounts of membrane-associated omega-3 fatty acids appears to reduce the susceptibility of the heart to ventricular fibrillation from ischemia. The prevailing scientific consensus is that omega-3 fatty acids exert their antiarrhythmic effects through multiple biological mechanisms including modulation of sodium and calcium channels and electrical stimulus threshold, inhibition of thromboxane production, lowering of nonesterified fatty acids in membranes and beneficial effects on heart rate variability.

7) Intervention Trials

Numerous studies have been conducted on the effects of dietary modification or supplementation with omega-3 fatty acids on multiple cardiovascular health indicators in patients with pre-existing cardiovascular disease and in normal human subjects. These intervention trials are summarized by chronological order in Table 9. In most of the intervention trials published through 1999, the intent of the omega-3 fatty acid supplementation was to determine if there was a therapeutic benefit on restenosis or progression of atherosclerosis in patients who had previously experienced a myocardial infarction or had undergone angioplasty associated with coronary atherosclerosis. With the exception of a modest effect on atherosclerosis reported by von Schacky *et al.*, (1999)⁵⁵, none of the intervention trials were able to show any beneficial effect on restenosis or progression of atherosclerosis by omega-3 fatty acid supplementation. Similarly, the study of omega-3 supplementation in patients with peripheral artery disease by Leng *et al.* (1998)⁵⁶ did not indicate any therapeutic effects.

In the early studies by Burr *et al.* (1989)⁵⁷, Reis *et al.* (1989)⁵⁸ and Kaul *et al.* (1992)⁵⁹, the lack of beneficial effects of omega-3 fatty acid supplementation on restenosis was reflected in the increased relative risk of nonfatal myocardial infarction in the treatment groups. However, in nine subsequent intervention trials in patients with coronary heart disease beginning with the study by Leaf *et al.* (1994)⁶⁰ and continuing to the report of Marchioli *et al.* (2002)⁶¹ as shown in Table 9, supplementation with omega-3 fatty acids was found to consistently reduce the relative risk of nonfatal myocardial

infarction, fatal myocardial infarction, sudden cardiac death and overall mortality associated with cardiovascular disease. The mean relative risk and range of relative risks found for treated groups in comparison to controls for these nine studies were as follows: nonfatal myocardial infarction (0.6, 0.3-0.9), fatal myocardial infarction (0.5, 0.2-0.8), sudden death (0.4, 0.1-1.0) and overall cardiovascular mortality (0.6, 0.2-0.8). In these high risk populations given capsules of EPA (0.3-6 g/day) and DHA (0.6-3.72 g/day) or ALA (2 g/day) in the diet, there was a strong association between the reduction of adverse coronary events and mortality and increased intake of omega-3 fatty acids. In fact, these results were so striking that in one study planned for a five year follow-up, patients on an ALA-rich Mediterranean diet (1 g/day omega-3 fatty acids) were observed to have a 70% reduction in cardiovascular deaths and complete suppression of sudden cardiac death at 27 months, so for ethical reasons, the study was terminated and all patients including controls were placed on the ALA-rich diet. Significantly lowered cardiovascular mortality (RR=0.59) was evident only three months into the study in treated groups.

Table 8. Case-control studies of relationship of omega-3 intake and blood/RBC levels and disease

Study reference	Study type		No. of subjects	Duration/ Follow-up (years)	Age range or mean	Outcome
Siscovick <i>et al.</i> , 2000	Case control study: Primary cardiac arrest patients matched with healthy controls	Dietary intakes of seafood by questionnaire; RBC fatty acid analysis	334 493 controls	NA	25-74	Moderate intake of omega-3 PUFA (5.5 g/mo or 1 fatty fish meal/wk) resulted in 50% reduction of risk of primary cardiac arrest; increased RBC long chain omega-3 PUFA was associated with a dose-related decrease in risk of cardiac arrest
Tavani <i>et al.</i> , 2001	Case control study in post-MI patients:	Intake of fish from dietary questionnaire to estimate omega-3 PUFA	507 478 controls	NA	25-79 61	Multivariate odds ratio of non-fatal MI at lowest omega-3 PUFA intake, 1.0, mid, 0.67; highest, 0.67
Albert <i>et al.</i> , 2002	Nested case-control study of sudden CVD deaths	Fatty acid analysis on 94 healthy males dying suddenly from CVD vs. 184 controls; blood fatty acid analysis	94 184 controls	17	40-82	Inverse relationship of long-chain omega-3 fatty acids in blood to risk of sudden death from CVD; association linear through quartiles and men with highest blood levels had 81% risk reduction in comparison to lowest LC-omega-3 levels.

Another consistent finding in these studies was a significant reduction in plasma triglycerides. Other findings that were variously reported in these intervention trials included reduction in systolic blood pressure, reductions in total cholesterol, increases in HDL-cholesterol and decreases in LDL-cholesterol. Singh *et al.* (1997)⁶² also reported that supplementation with EPA and DHA or ALA in mustard oil reduced cardiac arrhythmias, left ventricular enlargement and angina pectoris in patients.

In addition to the intervention trials in high risk patients, short term studies on healthy volunteers (Andrioli *et al.*, 1999)⁶³ have shown beneficial effects from omega-3

supplementation on platelet adhesion. As summarized earlier, humans who ingested omega-rich eggs had lowered plasma triglycerides, and in one study, HDL-cholesterol was significantly increased. Plasma total cholesterol and LDL-cholesterol were increased in controls eating standard eggs, but were essentially unchanged by intake of eggs with omega-3 fatty acids enrichment (Jiang and Sim, 1993; Prado-Martinez et al., 2003; Watrin et al., 2003)^{13 14 15}.

In summary, the findings of the intervention trials strongly support the cardioprotective effects of omega-3 fatty acids, including EPA, DHA and ALA, in secondary protection from coronary heart disease and cardiovascular mortality in high risk populations. In a recent review, Hu et al., (2001)⁶⁴ attribute these results to multiple mechanisms associated with omega-3 fatty acids. These are primarily reduction in triglyceride levels, reduction in platelet aggregation and antiarrhythmic effects. Additional factors may be an improvement in endothelial dysfunction and improved endothelial-dependent vasomotor function.

Table 9. Intervention trials with dietary modification and/or omega-3 dietary supplementation

Study reference	Study type	Treatment Regimen	No. of subjects	Duration/ Follow-up (years)	Age range or mean age	Outcome
Turpeinen et al., 1979 ⁶⁵	Intervention trial	Serum cholesterol lowering (SCL) diet (P/S ratio (0.48) versus normal diet (P/S ratio 0.25)	676	4.5	34-64	Approximately 50% reduction in CHD incidence associated with SCL and higher polyunsaturated /saturated (P/S) fat ratio
Burr et al., 1989 (DART)	Intervention trial in post-MI patients	EPA, 0.5 g/d; fish 200-400 g/2x/wk; or EPA/DHA 1.5 g/d in 3 capsules	1015	2	Under 70	Relative risk of non-fatal MI 1.5; fatal MI, 0.7; overall mortality, 0.7
	Control	No advice on fish consumption	1018	2		

Table 9. Intervention trials with dietary modification and/or omega-3 dietary supplementation

Study reference	Study type	Treatment Regimen	No. of subjects	Duration/ Follow-up (years)	Age range or mean age	Outcome
Reis <i>et al.</i> , 1989	Intervention trial in coronary angioplasty patients	EPA, 6 g/d; DHA, 3.72 g/d, given in 12 capsules	137	0.5	69	Relative risk of non-fatal MI, 6.9; relative risk of restenosis 1.7 for fish oil treated patients
	Control	No intervention	67	0.5		
Kaul <i>et al.</i> , 1992.	Intervention trial in coronary angioplasty patients	EPA, 5.4 g/d; DHA, 3.6 g/d, given in 10 capsules	58	0.5	56	Relative risk of non-fatal MI, 1.5; no effects on restenosis associated with omega-3 supplementation
	Placebo control	Corn oil	49	0.5	59	
Jiang and Sim, 1993	Normal healthy males	2 eggs containing 1170 mg omega-3 fatty acids	12	18 days	18-32	Significant reduction in plasma triglycerides and increase in plasma HDL cholesterol. Plasma total cholesterol and LDL cholesterol rose in controls but unchanged in enriched egg group. Plasma lipid content of omega-3 fatty acids increased in enriched egg group.
	Normal healthy male controls	2 eggs/day with 250 mg omega-3 fatty acids	12	18 days	18-32	

Table 9. Intervention trials with dietary modification and/or omega-3 dietary supplementation

Study reference	Study type	Treatment Regimen	No. of subjects	Duration/ Follow-up (years)	Age range or mean age	Outcome
Leaf <i>et al.</i> 1994.	Intervention trial in coronary angioplasty patients	EPA, 4.1 g/d; DHA, 2.8 g/d, given in 10 capsules	226	0.5		Relative risk of fatal MI, 0.5; sudden death, 0.5; overall mortality, 0.2; no effects on restenosis associated with omega-3 supplementation
	Placebo control	Corn oil	221	0.5		
Sacks <i>et al.</i> , 1995. ⁶⁶	Intervention trial in coronary angioplasty patients	EPA, 2.9 g/d; DHA, 1.9 g/d, given in 12 capsules	31	2.3	30-75	Relative risk of non-fatal MI, 0.5; fatal MI, 0.4; overall mortality, 0.4; no beneficial changes in atherosclerosis of the coronary arteries
	Placebo control	Olive oil	28	2.3		
Singh <i>et al.</i> , 1997	Intervention trial in post-MI patients	EPA, 1.08 g/d; DHA, 0.72 g/d, given in 6 fish oil capsules or 2.9 g/d ALA in mustard oil	122 120	1	49	Relative risk of non-fatal MI, 0.5; fatal MI, 0.6; sudden death, 0.2; overall cardiac mortality, 0.5 for fish oil supplements; similar reductions with ALA; significant reduction in triglycerides with fish oil only. Both treatments reduced cardiac arrhythmias, left ventricular enlargement and angina pectoris
	Placebo control	Al(OH) ₃ ; 0.1 g/d	118	1		

Table 9. Intervention trials with dietary modification and/or omega-3 dietary supplementation

Study reference	Study type	Treatment Regimen	No. of subjects	Duration/ Follow-up (years)	Age range or mean age	Outcome
Leng <i>et al.</i> , 1998.	Intervention trial in coronary angioplasty patients	GLA, 1.68 g/d; DHA, 0.27g/d, given in 6 capsules	60	2	65	Relative risk of non-fatal MI, 0.8; overall mortality, 1.0; no effects on peripheral artery disease
	Placebo control	Sunflower oil, 0.5 g/d	60	2		
GSSI Prevenzione, 1999 GSSI-1 (Stone 2000) ⁶⁷	Intervention trial in post-MI patients	EPA, 0.3 g/d; DHA, 0.6 g/d, given in 1 capsule; 300 mg/d Vitamin E or both in combination	2830/ group	3.5	NS	Relative risk of non-fatal MI, 0.9; fatal MI, 0.8; sudden death, 0.6; overall mortality, 0.8 in patients given omega-3 PUFA; no effect of Vitamin E on mortality. No change in blood lipid profile other than significant decrease in triglycerides in omega-3 treated group
	Control:	No intervention	2828	3.5		
Von Schacky <i>et al.</i> , 1999	Intervention trial in coronary angioplasty patients	EPA, 1.06 g/d; DHA, 0.65 g/d, given in 3 capsules	111	2	58	Relative risk of non-fatal MI, 0.4; fatal MI, 0.5; sudden death, 0.7; overall mortality, 0.5; modest mitigation of the course of coronary artery atherosclerosis
	Placebo control	Fatty acid mixture	112	2	58	

Table 9. Intervention trials with dietary modification and/or omega-3 dietary supplementation

Study reference	Study type	Treatment Regimen	No. of subjects	Duration/ Follow-up (years)	Age range or mean age	Outcome
De Lorgeril <i>et al.</i> , 1999 ⁶⁸	Intervention trial in post-MI patients:	Mediterranean diet rich in ALA, ~ 2 g/d	219	3.9	NS	Relative risk of non-fatal MI, 0.3; fatal MI, 0.3; sudden death, 0.1; overall mortality, 0.6
	Controls	Dietary advice by hospital dietitian	204	3.9		
Andrioli <i>et al.</i> , 1999	Healthy volunteers	20 ml fish oil with 0.3 g/d omega-6/3.6 g/d omega-3; ratio 0.1; or soy lecithin 1.5 g/d omega-6/0.5 g/d omega-3, ratio 3	10/sex/group; 60 total	15 days	25-45	Significant decreases in platelet adhesion with ADP and thrombin in fish oil group; platelet adhesion increased in lecithin group; correlation between lower omega-6/omega-3 ratio and reduced platelet adhesion.
Marchioli <i>et al.</i> , 2002 GSSI-2 ⁶⁹	Intervention trial on post-MI patients	1 g/d omega-3, 300 mg/d Vitamin E, both combined	5666	3.5	59	Relative risk of non-fatal MI, 0.91; non-fatal stroke, 1.22; sudden death, 0.55, cardiac/coronary death, 0.65/0.68, CVD death, 0.70.
	Controls	No treatment	5657	3.5		

Table 9. Intervention trials with dietary modification and/or omega-3 dietary supplementation

Study reference	Study type	Treatment Regimen	No. of subjects	Duration/ Follow-up (years)	Age range or mean age	Outcome
Prado-Martinez <i>et al.</i> , 2003	Intervention dietary trial in Spanish normal post-menopausal women:	1.13 Columbus eggs/d with 725 mg/egg for both omega-3 and omega-6 fatty acids; signed voluntary informed consent forms	40	0.16	45-60	10% reduction in triglycerides, 20% reduction in insulin, 6% decrease in systolic blood pressure and 1% weight loss in treated vs. controls; no change in cholesterol, blood glucose, LDL or HDL cholesterol
	Controls:	0.65 standard egg/d on average; signed voluntary informed consent forms	40		45-60	No notable changes in any parameter evaluated
Watrin <i>et al.</i> , 2003	Intervention trial in adolescents with hypercholesterolemia:	2-4 Columbus eggs/week for 8 weeks (Hospital Ethics Board reviewed)	12	0.16	Adolescents	18% decrease in triglycerides and 6.5% in Apo A-1; otherwise negligible effects on lipid factors such as cholesterol, LDL- or HDL-cholesterol, or Apo B

C) ADDITIONAL ISSUES TO ADDRESS IN QUALIFIED HEALTH CLAIM PETITIONS

1. Omega-3 fatty acid-enriched eggs will provide a meaningful amount of omega-3 fatty acids in the diet.

The scientific evidence presented in this review clearly supports a qualified health claim for omega-3 fatty acid enriched eggs for their cardio-protective properties. To date, FDA has not yet established a daily value for omega-3 fatty acids. An omega-3 fatty acid-enriched egg should be eligible to bear the contemplated qualified health claim because it does provide 660mg omega-3 fatty acids per RACC (reference amount customarily consumed). FDA itself on October 31, 2000 (Docket No. 91N-0103) used its enforcement discretion to allow a qualified health claim about EPA and DHA omega-3 fatty acids in dietary supplements and reduced risk of coronary heart disease.

The IOM expert panel recently established an AMDR for Alpha-linolenic acid at 0.6 to 1.2 percent of energy and recognized that up to 10 percent of this range can be consumed as EPA and/or DHA. The lower boundary of the AMDR is based on the AI for alpha-linolenic acid, which is 1.1 and 1.6 g/day for women and men, respectively. The recommended intake of DHA and /or EPA on the lower end of the AMDR, therefore, is 110 to 160 mg. (www.nap.edu/openbook/0309085373/html/609.html)

The omega-3 fatty acid enriched eggs that are the subject of this petition contain 660 mg of omega-3 fatty acids per 50g edible egg portion of which 550 mg is ALA, 10 mg EPA, 20 mg DPA and 80 mg DHA and clearly provide a meaningful amount of omega-3 fatty acids in the diet.

2. Public Health Benefit

The public health of the general population stands to benefit from the proposed claim in that authorization of the claim for an omega-3-fatty acid enriched egg is

expected to lead to increased consumption of the omega-3 fatty acids which is highly desirable. There is considerable scientific evidence that their increased intake will reduce the risk of CHD, a serious health concern that is estimated to result in the deaths of millions of Americans each year and impose billions of dollars in associated health care costs (www.fda.gov/fdac/203_toc.html). Dr. Mark McClellan, Commissioner of Food and Drugs has publicly stated that omega-3 fatty acids are an ideal candidate for a qualified health claim. The Office of Management and Budget (OMB) similarly noted that “epidemiological and clinical studies find that an increase in consumption of omega-3 fatty acids results in reduced deaths due to CHD” (www.whitehouse.gov/omb/inforeg/prompt_dietary_052703.pdf). Dr. John D. Graham of OMB has requested in letters to both the Honorable Claude A. Allen, Deputy Secretary of the Department of Health and Human Services as well as the Honorable James R. Moseley, Deputy Secretary of the Department of Agriculture, revisions to the “Dietary Guidelines” and the “Food Guide Pyramid” noting the significant potential improvement in public health suggested by the current science on the importance of reducing foods high in trans fatty acids and increasing consumption of foods rich in Omega-3 fatty acids. The omega-3 fatty acid –enriched egg that is the subject of this petition clearly fits into these types of foods. Other experts including the American heart Association also recognize the relationship between omega-3 fatty acids and a reduced risk of CHD.

As a natural constituent of many foods, omega-3 fatty acids are considered safe for consumption and their presence in foods has not been reported to cause any adverse health effects. Greenland Eskimos consumed an average of 13.7 g/day of omega-3 fatty acids without reports of adverse effects. No significant adverse effects were reported in the scientific literature in clinical studies where omega-3 fatty acids were given for prolonged periods of time at doses in excess of 10 g/day. Mild side effects such as diarrhea, prolonged bleeding time, nosebleeds and “fishy-smelling” breath, skin and urine have been reported in individuals consuming high doses of omega-3 supplements. No adverse effects are anticipated from consumption of eggs containing 660 mg omega-3 fatty acids/egg.

There does not appear to be an optimal level of omega-3 fatty acid intake. Substantial reductions in cardiovascular risk have been achieved with doses as low as 200 mg to 1 g/day or 1-2 g/week in human studies. Other studies of blood and membrane composition have shown an inverse linear relationship of coronary sudden death risk and percentage of omega-3 fatty acid composition without a defined upper limit of benefit.

Omega-3 fatty acids are ingested at levels up to 660 mg/egg in the eggs that are the subject of this petition; intake at these levels in eggs poses no safety or health concerns and no special considerations are necessary to protect any segment of the population such as children, the elderly, or persons with any known medical conditions.

We do not expect any substantial changes in eating habits of eggs are expected in the U.S. population from the introduction of enhanced omega-3 eggs and no negative consequences would result in the total diet. The beneficial effect of a reduction in risk of cardiovascular disease is likely to result from consumption of one enhanced Columbus[®] or Christopher[®] egg/day with 660 mg of omega-3 fatty acids. As a point of reference, a standard 50 g egg contains 37.5 mg of omega-3 fatty acids (Agriculture Handbook No. 8, 1989)⁷⁰ and USDA Nutrient Database for Standard Reference, Release 12 (1998)⁷¹ on www.aeb.org and www.nal.usda.gov/fnic/cgi-bin/nut_search.pl.

D) SUMMARY AND CONCLUSIONS

As the preceding review demonstrates, the preponderance of evidence from publicly available scientific studies strongly supports the association of increased dietary intake of omega-3 fatty acids with reductions in the risk of cardiovascular disease and mortality from nonfatal and fatal myocardial infarctions, sudden death associated with arrhythmia and ventricular fibrillation and secondary coronary heart disease mortality. There is a substantial body of scientific evidence that establishes the biological plausibility and mechanisms for the prevention or mitigation of the pathophysiological

conditions leading to cardiovascular disease from a balanced intake of omega-3/omega-6 fatty acids. The scientific literature clearly demonstrates the beneficial physiological and biochemical effects of omega-3 fatty acids on inflammation, cardiac arrhythmia, thrombosis, lipid profiles, endothelial function and modulation of eicosanoid production and intensity. Epidemiology studies in human populations have shown a low incidence of cardiovascular disease in populations consuming increased amounts of omega-3 fatty acids that shift the intake ratios of omega-6 to omega-3 to lower values. Furthermore, case-control studies indicate there is an apparent inverse dose-response relationship in the risk of coronary heart disease and percentage of cell membrane or blood levels of omega-3 fatty acid content.

The published prospective cohort studies provide a consistent and convincing body of evidence that increased intake of omega-3 fatty acids from fish, oils, nuts and other dietary sources can significantly decrease the risk of sudden cardiac death, total mortality from coronary heart disease and the progression of coronary heart disease. In intervention trials, omega-3 fatty acid dietary supplementation has resulted in dramatic reductions, generally on the order of 50%, in nonfatal and fatal myocardial infarctions, sudden cardiac death and overall coronary heart disease mortality, that are unmatched by any other dietary supplement for their consistent and significant beneficial effects in high risk patients.

As previously noted, the American Heart Association estimates that approximately 62 million Americans or 1 in 5 men and women have some form of cardiovascular disease. Thus, cardiovascular disease is by far the leading cause of mortality and illness in the United States. In 2000, nearly 40% of all deaths were associated with cardiovascular disease (946,000/year or 2600/day). Clearly, the significant benefits of increased omega-3 fatty acid consumption and a restoration of a better balance in the diet between omega-3 and omega-6 intakes demonstrated by the scientific evidence are applicable and will be of public health benefit to the general U.S. population in an overall reduction of cardiovascular disease and mortality. Harper and Jacobson, (2003)⁷² in a recent review addressed the current consumption of omega-3 fatty acids and proposed dietary recommendations. The average US intake of omega-3 fatty

acids is about 1.6 g/day (about 0.7 % of a 2200 calorie diet). While no official recommendations for omega-3 fatty acid intake have been made in the USA, an expert panel of nutritionists has suggested some guidelines of 2.85 g/day total omega-3 fatty acids comprising 2.2 g/day of ALA and 0.65 g/day of EPA plus DHA. Similar recommendations have been made by the British Nutrition Foundation as well as several other international health organizations. For this to occur, ALA intake would need to increase to 2.2 g/day from about 1.4 g/day (a 57 % increase) and EPA and DHA intake would need to increase from 0.2 g/day to 0.65 g/day (a 400 % increase) in order to meet these recommendations. While primary interventions trials are needed before recommending large changes in the food supply on consumption of omega-3 fatty acids, the authors conclude that even small increases could lead to large CHD event reductions in secondary prevention and the impact on health will be significant.

The claim of public health benefit from increased dietary intake of omega-3 fatty acids and a better balance between omega-3 and omega-6 fatty acids is supported by a growing scientific consensus among experts who have investigated the relationship between polyunsaturated fatty acid intake and heart disease. In 1999, an international group of experts in this field met for a Workshop on the Essentiality of and Recommended Dietary Intakes (RDIs) for Omega-6 and Omega-3 Fatty Acids at the National Institutes of Health, Bethesda, MD (Simopoulos et al 1999)⁷³. Consensus was reached on reducing intake of omega-6 fatty acids and increasing omega-3 fatty acids in the diet. Therefore, the recommended adequate intakes of linoleic acid (LA) were set at 4.44 g/day or 2% of total energy intake with an upper limit of 6.67 g/day or 3% of a 2000 kcal diet. The adequate intake of ALA was set at 2.22 g/day or 1% of energy intake for a 2000 kcal diet. Adequate intakes of DHA plus EPA were set at 0.65 g/day (min. 0.44 g/day) and 0.3% (min. 0.2%) of energy intake.

The importance of increased omega-3 fatty acids in the diet and mitigation of cardiovascular disease has been recognized by other authoritative groups. The Nutrition Committee of the American Heart Association in 2003 recommended that persons without coronary heart disease should consume a variety of preferably oily fish at least twice a week and include foods rich in ALA; patients with coronary heart disease should consume 1 g/day of EPA and DHA and patients requiring lowering of plasma

triglycerides should consume 2-4 g/day of EPA and DHA. The USDA has recently proposed that polyunsaturated fatty acids be included in the listing of nutritional components of the Food Guide Pyramid (68 FR 53536, September 11, 2003)³. The nutritional goals for proposed daily food intake patterns include linoleic acid and alpha-linolenic acid for specified levels of daily caloric intake. For example, Adequate Intakes (AI) of linoleic acid and alpha-linolenic acid for a 2400 calorie diet are 17 and 1.6 g/day, respectively and the Acceptable Macronutrient Distribution Ranges (AMDR) are 5-10% and 0.6-1.2% of total caloric intake for linoleic acid and alpha-linolenic acid.

Finally, Hu and Willett, (2002)⁷⁴, conducted an extensive search of the scientific literature through May 2002 for epidemiologic and clinical investigations of major dietary factors (fat, cholesterol, omega-3 fatty acids, trans-fatty acids, carbohydrates, glycemic index, fiber, folate, specific foods and dietary patterns) and their association with CHD. After a review of 147 original investigations, they concluded that substantial evidence exists to support three dietary strategies for preventing CHD: substitute nonhydrogenated unsaturated fats for saturated fats and trans-fats; increase consumption of omega-3 fatty acids from fish, oil supplements, or plant -derived sources; and consume a diet high in vegetables, nuts and whole grains and low in refined grain products. There clearly is a positive health benefit opportunity for omega-3 fatty acid-enriched eggs as a part of selecting one's diet to contain a good source of omega-3 fatty acids. This also includes the ability to increase the intake of omega-3 fatty acids to a more desirable dietary level, improving the omega-3 fatty acids to the omega-6 fatty acids ratio in the diet as well as providing a more convenient source for omega-3 fatty acids than as a dietary supplement.

E) **HEALTH CLAIM STATEMENT**

Petitioners propose that the qualified health claim be approved for labeling of the Belovo Columbus[®] or Christopher[®] eggs with increased levels of omega-3 fatty acids as follows:

Consumption of one egg per day containing 660 mg of omega-3 fatty acids with a balanced ratio of omega-3 to omega-6 fatty acids (1:1) may reduce the risk of heart disease and sudden, fatal heart attack. FDA has determined that the scientific evidence is supportive, but not conclusive, for this claim. See nutrition information for cholesterol content.

As presented in the scientific summary, both the emerging scientific consensus of experts in nutrition and cardiovascular disease and authoritative bodies such as the American Heart Association and USDA have recognized the consistent and convincing scientific evidence for omega-3 fatty acid's role in reducing the risk of coronary heart disease and mitigation of sudden, fatal heart attacks by recommending increases in dietary omega-3 fatty acid intake. As consumed in a conventional food, omega-3 enhanced egg products offer the general public a means to better nutritional and health status without noticeable alteration in current eating habits and preferences.

F) EXPERT PANEL CONSENSUS STATEMENT

The undersigned, an independent panel of recognized experts qualified by their scientific training and relevant national and international experience to evaluate the safety of food and nutritional ingredients, was requested by Belovo S.A. to review its qualified health claim petition and determine if the proposed qualified health claim is supported by the scientific evidence and that the scientific evaluation represents a reasonable and balanced representation and interpretation of the pertinent scientific literature. The Scientific Advisory Board independently evaluated the petition and other materials deemed appropriate or necessary. Following independent, critical evaluation, the Scientific Advisory Board conferred and unanimously agreed to the decision described below.

Based upon a critical review and analysis of the information summarized herein, the Expert Panel determined that the scientific evidence in the published literature supports the qualified health claim made for enhanced omega-3 fatty acid content of 660 mg/egg with a balanced ratio of omega-6 to omega-3 fatty acids of approximately one and reduction of the risk of heart disease. Further, the Expert Panel considers that the petition presents a reasonable and balanced representation and interpretation of the pertinent scientific literature with regard to the potential health benefits of omega-3 fatty acids in the diet.

Andrew Sinclair 23 December 2003
Andrew J Sinclair, Ph.D. Date

Michel de Lorgeril December 23, 2003
Michel de Lorgeril, M.D. Date

W. Gary Flamm Dec. 22, 2003
W. Gary Flamm, Ph.D., FATS Date

G. ANALYTICAL METHODS/RESULTS

The analytical data for cholesterol, saturated fat, monounsaturated fat, and polyunsaturated fats, specifically for both omega-3 and omega-6 fatty acid composition is given in Attachment B. The analytical method for conducting this analysis is AOAC Official Method 969.33 Fatty Acids in Oils and Fats. Section 41.1.28-Oils and Fats. The assay method can be utilized with eggs. The analytical results for Christopher and Columbus eggs omega-3 fatty acid content are found in Attachment C.

H. ATTACHMENTS

Copies of the bibliography of scientific articles as well as each referenced publication can be found in Attachment D as well as any other materials referenced to herein or that are part of the basis for this petition. To the best of Petitioner's knowledge, all non-clinical studies were conducted in compliance with good laboratory practice regulations set forth in 21 CFR Part 58 or were exempt from such regulations at the time of conduct or were conducted for experimental research purposes only in academic facilities and not expressly intended for formal FDA submission. To the best of Petitioner's knowledge, all clinical studies were conducted in compliance the requirements for institutional review set forth in 21 CFR Part 56 or were not subject to such requirements in accordance with 21 §CFR 56.104 or 56.105 and were conducted in conformance with the requirements for informed consent set forth in 21 CFR §50.

I. ENVIRONMENTAL IMPACT

The Petitioner claims that the petition and claim is categorically excluded from having to prepare an environmental assessment (EA) or environmental impact statement (EIS) under 21 CFR §25.32(p), categorical exclusions from EA's or EIS on foods, food additives and color ingredients for issuance of a health claim petition. To the best of the Petitioner's knowledge, the subject petition and substance are in compliance with and regulated by the provisions of 21 CFR §25.32(p) and no extraordinary circumstances exist that would require a submission of an EA.

J. CONCLUSION AND CERTIFICATIONS

The petitioner requests that the FDA utilize its enforcement discretion and grant the Petitioner the use of the model qualified health claim for labeling purposes on its Columbus[®] or Christopher[®] enhanced omega-3 egg products.

Pursuant to 21 CFR§101.70(h), we certify that, to the best of our knowledge, the petition and scientific summary are representative and balanced submissions that include unfavorable information as well as favorable information, known to us to be pertinent to the evaluation of the proposed health claim.

Stanley M. Tarka, Jr. December 23, 2003
Stanley M. Tarka, Jr., Ph.D. Date
Petition Author

Michael Hawes Dec. 15, 2003
Michael Hawes Date
President, Belovo, Inc., U.S.A.
Authorized Company Official

On behalf of: [Signature] 15.12.03
Fabien De Meester, Dr. Date
President & CEO, Belovo, S.A.
Authorized Company Official

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