Omega 3-Fatty Acids: Health Benefits and Cellular Mechanisms of Action

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Abstract: Epidemiological evidence has established that ingestion of long-chain polyunsaturated omega-3 fatty acids (ω-3 PUFAs), abundant in fish oils, have profound effects on many human disorders and diseases, including cardiovascular disease and cancer. Here we briefly review the dietary recommendations and the food sources that are naturally enriched by these fatty acids. There are also a number of products including eggs, bread, and cereals available to supplement ω-3 fatty acid dietary intake. Some of these supplements are proposed to aid different pathological conditions. While the beneficial effects of omega-3 fatty acids can no longer be doubted, their molecular mechanism of action remains elusive. Without question, the action of omega-3 fatty acids is complex and involves a number of integrated signaling pathways. This review focuses on one of the possible cellular mechanisms by which the ω-3 PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), may function. Studies with cancer cells suggest that DHA induces cell cycle arrest and apoptosis by activating protein phosphatases, leading to dephosphorylation of retinoblastoma protein (pRB). Protein phosphatases are also involved with the protein Bcl2, which regulates the release of cytochrome c from mitochondria, and eventually, activation of the apoptotic enzyme caspase 3.

Keywords: Omega-3 fatty acids, Cancer, Dietary intake, Food supplements.

INTRODUCTION

It is now evident that "all fats are not created equal". Some fats, such as cholesterol, saturated fats and polyunsaturated long-chain omega-6 fatty acids, taken in excess are considered "bad" for human health while the class of long-chain polyunsaturated omega-3 fatty acids (ω-3 PUFAs) are beneficial. Table 1 presents a partial list of human afflictions that have been alleviated by ω-3 PUFAs. It is unclear how this class of very simple molecules can affect so many seemingly unrelated diseases. The reasons behind the beneficial properties of the omega-3 fatty acids are the subject of considerable interest and intense investigation.

EPIDEMIOLOGY STUDIES

The first clue that ω-3 PUFAs may exert beneficial effects on human health came from epidemiology studies on populations in which fish was a major component of the diet. The favorable health effects of ω-3 PUFAs on the cardiovascular system was initially recognized by Dyerberg *et al.* in the 1970s [1]. These researchers observed that Greenland Eskimos, who consumed a diet rich in ω-3s, had a low rate of cardiovascular disease as measured by a number of factors. Similar observations were also made for a Japanese fishing village that consumed an average of 250g of fish daily compared to a Japanese farming village that only averaged 90g of fish daily [2]. There are now many studies that have found an inverse association between fish oil consumption and risk of coronary heart disease (CHD) or sudden cardiac death in the general population [3-7]. In addition to the beneficial cardiovascular effects, use of fish oil was also reported to have anticancer properties. An epidemiology study of South African West Coast fisherman reported that despite smoking; high sodium intake; low consumption of fiber, fruits, and vegetables; absence of vitamin supplementation; and low levels of dietary micronutrients, compared to urban whites, the fisherman had a lower incidence of colorectal cancer [8]. This was attributed to the protective effects of fish oil in their diets [8]. Similarly, a population-based case-control epidemiology study in Norway demonstrated an inverse relationship between serum ω-3 PUFA concentrations and thyroid cancer [9]. Cross-national studies have shown an inverse relationship between fish consumption and incidences of and mortality rates from prostate [10, 11] and breast cancer [12-16]. Furthermore, a series of case-controlled studies in Italy and Switzerland suggest that ω-3 PUFAs decrease the risk of several cancers, including oral and pharyngeal, esophageal, colon, breast, and ovarian cancers [17].

OMEGA-3 FATTY ACIDS AND THE DIET

Soon after the original epidemiology studies on fish oil diets became appreciated, it was evident that the "Western diet," rich in saturated fats, was partially responsible for the high incidence of cancer and heart disease associated with modern societies. An emphasis was then placed on substituting animal fats with unsaturated vegetable oils from corn, sunflower seeds, safflower seeds, cottonseed, and soybeans. Since these oils are rich in ω-6 fatty acids, there has been an associated increase in the ω-6/ω-3 dietary lipid ratio in Western societies. The ω-3 and ω-6 families of PUFAs function differently because of the location of the first double bond in the 3rd (omega-3) or 6th (omega-6)

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Table 1. Reported Beneficial Effects of ω-3 PUFAs in Various Diseases

Disease/Disorders	Reference
ADHD	[135]
Aggression	[136]
Alcoholism	[137]
Arthritis	[138]
Asthma	[139,140]
Bipolar Disorder	[141]
Blindness	[142]
Cancer	[9,17,88,143-149]
Crohn's Disease	[150]
Cystic Fibrosis	[151]
Depression	[152]
Dermatitis	[153]
Dyslexia	[154]
Heart Disease	[155,156]
Hypersensitivity	[157]
Kidney Disease	[158]
Lupus Erythematosus	[159]
Malaria	[160]
Migraine Headaches	[161]
Multiple Sclerosis	[162]
Neurovisual Developmental Disorders	[163]
Nephropathy	[164]
Peroxisome Biogenesis Disorder	[165]
Phenylkenonuria	[166]
Psoriasis	[167]
Respiratory Diseases	[168]
Schizophrenia	[169]
Suicide	[152]
Ulcerative Colitis	[26,27]

positions from the methyl terminal of the aliphatic carbon chain (Fig. (**1**)). In a typical Western diet, the ratio of ω-6 to ω-3 fatty acids now ranges from approximately 20-30:1 instead of the range of 1-2:1, which is believed to have been present in the diets of prehistoric populations that survived on fresh fruits, vegetables, fish, and animals [18]. A similar low ratio of ω-6/ω-3 dietary lipids has been reported for modern populations subsisting on a fish-based diet [1, 19]. Corresponding to this dramatic change in PUFA dietary ratio is an increased risk of cardiovascular, cancer, and other diseases among Western populations compared to populations that lived before the Industrial Revolution and those currently living on diets rich in fish oils [8, 9, 20-22]. The beneficial effects of fish oils are mostly attributed to their content of the ω-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

The 18-carbon ω-3 PUFA, α-linolenic acid, found in green leafy vegetables, flaxseed, rapeseed, and walnuts, can be desaturated and elongated in the human body to EPA and then to DHA. Therefore α–linolenic acid may have similar beneficial effects on health as the longer chain PUFAs. The ω-3 and ω-6 fatty acid families are important for human nutrition. The precursors of these fatty acids, 18-carbon linoleic (ω-6) and α-linolenic (ω-3), cannot be produced in the body and are therefore "essential" to the diet. Linoleic acid and linolenic acid are converted to longer chain ω-6 and ω-3 fatty acids by various cycles of desaturation and elongation, as presented in Fig. (**2**).

The most common food sources of the long-chain PUFA ω-3 fatty acids are cold-water fatty fish, including mackerel, salmon, herring, trout, sardines, and tuna (Table 2). Eggs and meat also contain small amounts of ω-3 fatty acids (Table 3). Increased intake of ω-3 PUFAs can also occur through consumption of dietary α-linolenic acid, which can be metabolically converted to EPA and DHA. Alpha-linolenic acid is a common component of flax seeds and canola oil. Flax seeds contain approximately 24% while canola oil contains approximately 11% α-linolenic acid. Canola oil is readily available in many foods such as bread and cereals, while energy bars often contain flax seed oils.

Because of the health benefits that ω-3 fatty acids provide, there is a need to set a Recommended Daily Allowance guideline for ω-3 fatty acids. As of yet, the United States Food and Drug Administration (FDA) has not made such a recommendation. In the typical Western diet, the average ω-3 fatty acid consumption is less than 0.1 gm/day. This is a very small amount considering that health authorities in Canada*, the United Kingdom†, and Australia‡ have made recommendations of 1-2 gm ω-3 PUFAs/day. In 1999, as part of an effort to evaluate the importance of ω-3 PUFAs, a workshop was held at the National Institutes of Health (Bethesda, Maryland, USA) to determine the recommended dietary intakes of ω-6 and ω-3 fatty acids. This workshop established an amount representing the Adequate Intake for Adults and Infants. For a 2000 kcal diet, the recommended intake of ω-3 fatty acids for adults was 0.65 gm/day, with a minimum intake of ω-3 fatty acids being 0.22 gm/day. The Adequate Intake for Infant Formula was set at approximately 2% of lipid intake [23]. More recently the American Heart Association recommended 1 gm/day of ω-3 PUFA for adults for the prevention of cardiovascular disease [24].

OMEGA-3 FATTY ACID SUPPLEMENTATION

Despite the fact that the Food and Drug Administration has yet to recommend precise dietary intakes of ω-3 and ω-6

* Nutrition Recommendations. In S.R. Committee, Ed.; Minister of National Health and Welfare: Ottawa, ***1990***; pp. H49.

† Unsaturated fatty acids-nutritional and physiological significance: the report of the British Nutrition Foundation's Task Force. In C.A. Hall, Ed.; The British Nutrition Foundation: London, ***1992***.

‡ Report of the NHMRC working party: the role of polyunsaturated fats in the Australian diet. ***1992.***

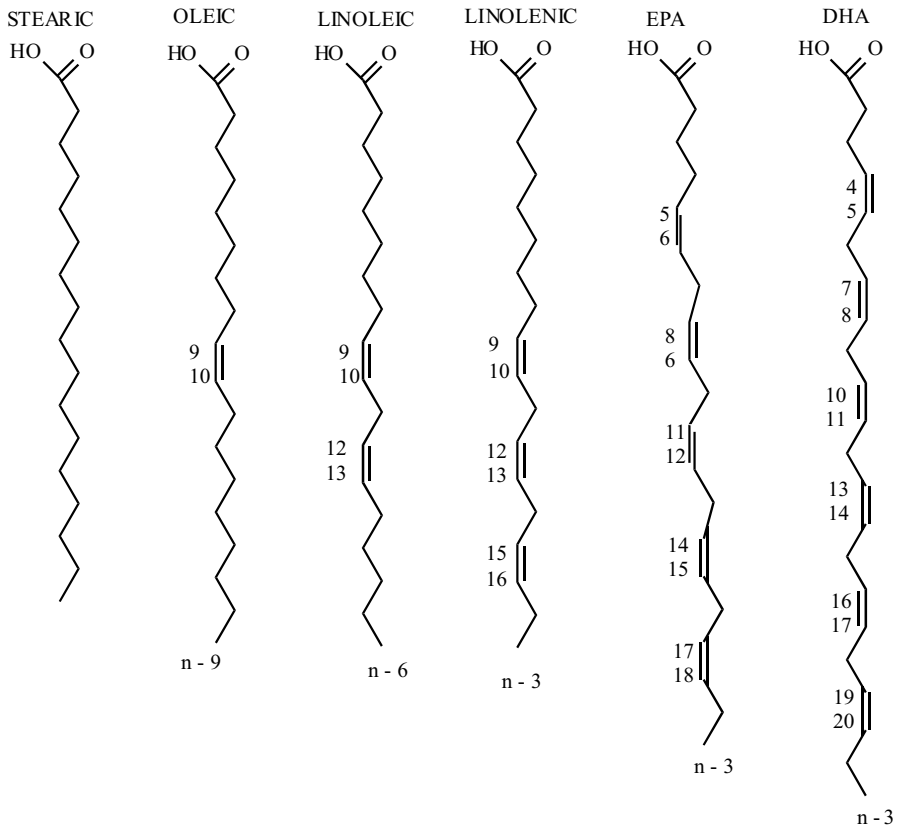

Fig. (1). Some biologically important fatty acids.

Fatty acids are classified in saturated, ω-3, ω-6, or ω-9 families based on the position of the last double bond at the 3rd, 6th, or 9th position from the methyl terminal of the aliphatic carbon chain.

PUFAs, there is little doubt that fatty acids can have a profound effect on human health. This realization has paved the way for increased marketing of ω-3 supplements and patents for future drug development.

There are hundreds of web sites advertising the availability of ω-3 products as well as countless formulas rich in ω-3 that have been patented. Most of the patents contain different mixtures of ω-3 and ω-6 fatty acids and are suggested for the treatment of a myriad of disorders (Table 4). Although it is beyond the scope of this review to thoroughly discuss the countless applications of the patented formulas, a few representative examples follow. Their uses range from enhancing the weakened immune system of trauma patients to combating the effects of aging caused by cigarette smoke. Some formulations comprise a liquid drink while others may be administered enterally to ICU patients who have depressed absorption capacity. Other patents include a method for restoring gut integrity as well as a formulation to combat the negative symptoms found in postmenopausal women.

Perhaps the most abundant area for development of ω-3 patents has been in the treatment of inflammatory diseases. Compositions containing ω-3 esters have been developed and clinically proven to treat psoriasis and phlebitis [25] as well as ulcerative colitis [26, 27]. For the treatment of chronic inflammation as well as liver disorders, a combination of various ω-3 fatty acids has been developed in the form of medium-length triglycerides. The administration of ω-3 fatty acids as medium-length triglycerides speeds clearance of the lipid emulsions from the blood and therefore provides certain advantages. Enhanced blood clearance of these lipids results from stimulation of specific tissue uptake and inhibition of the synthesis of endogenous triglycerides. Thus, the uptake of ω-3s as medium-length triglycerides may also contribute to an overall reduction of blood triglycerides [28]. Furthermore, accompanying ω-3 PUFAs with medium-length triglycerides protects them from rapid oxidation, and this combination alone has a protective effect on the liver [29, 30].

Because of the increased research involving the health benefits of ω-3 fatty acids, several functional foods have been designed to enhance ω-3 PUFA intake (Table 5). Two functional foods have been designed for very specific purposes. For infants, Mead Johnson Nutritionals (Bristol-Myers Pharmaceuticals, Evansville, IN) makes Enfamil Lipil™ brand baby formula, with levels of ω-3 PUFAs comparable to breast milk and significantly higher than other commercial formulas. DHA in infants is important because it is essential for proper brain and eye development. For cancer patients, Ross Pharmaceutical (Abbott Laboratories, Columbus, OH) makes Prosure, a nutrition and energy beverage containing DHA designed to help people reverse tumor-induced weight loss. Omega-3 PUFA intake may be increased by the use of ω-3 PUFA-enriched eggs, manufactured by many companies across Canada and the United States.

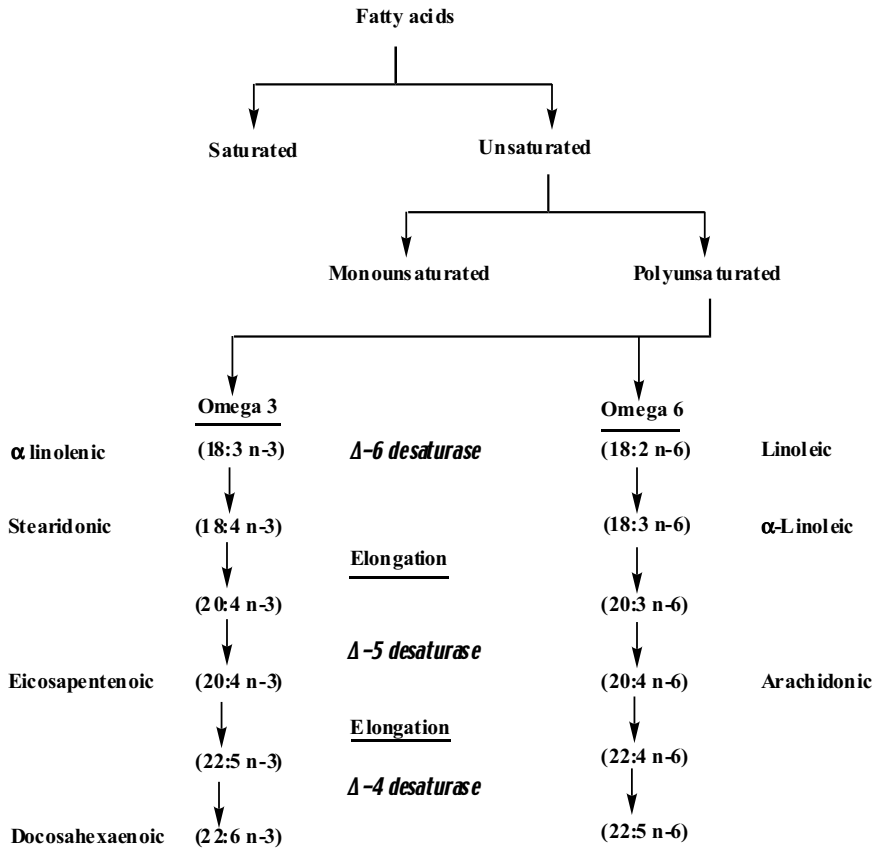

Fig. (2). Metabolic pathway of omega-6 and omega-3 fatty acid synthesis.

Fatty acids are classified as saturated or unsaturated fatty acids depending on the presence of double bonds. The unsaturated fatty acids are further divided into monounsaturated or polyunsaturated fatty acids. The polyunsaturated fatty acids are either ω-3 or ω-6 fatty acids. Alpha-linolenic acid and linoleic acid are the precursors of ω-3 and ω-6 fatty acids, respectively, and are converted to different long-chain polyunsaturated fatty acids by sequential desaturation and elongation.

We analyzed seven different brands of ω-3 fish oils (Sundown Flax Seed Oil, Sundown Cod Liver Oil, Sundown Fish Oil, Puritan's Pride Super EPA, Nature Made Fish Oil, Member's Mark Fish Oil, and Sigma-Aldrich Fish Oil) for their chemical composition (Table 6). It is clear from the labels of ingredients that different ω-3 supplements contain different amounts of ω-3 PUFAs ranging from 160 to 550 mg/g of oil. Our gas chromatographic analysis data further indicate some differences in quantities of ω-3 PUFA in the capsules versus the amount that is marketed by the manufacturers. These discrepancies could be due to the fact that the oils are extracted from fish that may be caught from different environmental conditions (cold water *vs* warm water) and in different seasons. These ω-3 PUFA supplements also have different levels of lipid peroxidation (data not shown) because each supplement may have been processed and stored differently as well as supplemented with different antioxidants. Furthermore, our data also indicate that fish oil supplements contain a significant quantity of other unsaturated fatty acids that nutrition labels neglect to reveal. While the appearance of other unsaturated fatty acids may have no effect on the ability of ω-3 PUFAs to function properly, the presence of other unsaturated fatty acids may have adverse biological effects. It is therefore important that these fatty acid supplements be evaluated and standarized against biological activites.

Because of the reputed ability of ω-3 fatty acids to support such a wide variety of health benefits, they could be considered as "wonder drugs". However, the FDA does not classify these nutritional compounds as drugs and the FDA does not officially recognize them as treatments for the diseases. Despite this fact, the patented formulas and marketed nutritional supplements that contain ω-3 lipids will most certainly continue to be developed.

A POSSIBLE MODE OF ACTION FOR OMEGA-3 PUFAs

Although epidemiological and nutritional evidence strongly suggest that ω-3 PUFAs have an influence on various disease states, the mechanism by which ω-3 PUFAs function remains unclear. Whatever omega-3 fatty acids' mode of action is, it must be fundamental and commonly shared by a wide variety of tissues. Several non-exclusive hypotheses regarding the mode of action of ω-3 PUFAs have been proposed. It has been suggested that ω-3 PUFAs may affect numerous membrane properties (e.g., permeability [31], "fluidity" [32], lipid packing [33], fusion [32], deformability [34] etc.); the activity of specific proteins

Table 2. ω-3 Fatty Acid (DHA and EPA) Content in Seafood[1]

Type	Amount of DHA (g/3oz portion)	Amount of EPA (g/3oz portion)	Amount Required to Provide ≈ 1 gm of DHA and EPA
Catfish			
Farmed	0.116	0.085	15 oz
Wild	0.109	0.042	20oz
Clams	0.124	0.177	10 oz
Cod			
Atlantic	0.131	0.003	23 oz
Pacific	0.147	0.088	13 oz
Crab, Alaskan King	0.100	0.251	9 oz
Flounder/Sole	0.219	0.207	7 oz
Halibut	0.318	0.077	8 oz
Lobster	0.026	0.045	42 oz
Oyster			
Eastern	0.496	0.456	3 oz
Farmed	0.179	0.195	8 oz
Pacific	0.425	0.745	3 oz
Salmon			
Atlantic Farmed	1.238	0.587	2 oz
Atlantic Wild	1.215	0.349	2 oz
Pink Canned	0.685	0.718	2 oz
Scallops	0.092	0.076	18 oz
Shrimp	0.122	0.145	11 oz
Trout, Rainbow			
Farmed	0.697	0.284	3 oz
Wild	0.442	0.398	4 oz
Tuna			
Canned, light	0.190	0.040	13 oz
Fresh	0.970	0.309	2 oz
White	0.535	0.198	4 oz

[1]USDA Nutrient Data Laboratory. Http://www.nalusda.gov/fnic/foodcomp/. Accessed August 5, 2003.

(e.g., protein kinase C [35], rhodopsin [33], (Na+, K+)-ATPase [36], and Na+ channel [37]); lipid micro domain formation [38-40]; eicosanoid biosynthesis [41]; gene expression [42]; and formation of potent lipid peroxidation products [43]. It is likely that a combination of several of these effects is responsible for the beneficial health properties of ω-3 PUFAs. Here we will describe one possible molecular action of omega-3 fatty acids, their role in programmed cell death (apoptosis).

Table 3. Foods Naturally Containing ω-3 PUFAs[2]

Food	Amount of ω-3 PUFA (mg)
1 large hard-boiled egg	19
2 pieces fried chicken	37
3 oz tuna salad	47
12 large steamed shrimp	96
1 cup chicken livers	112
3 oz steamed crab	196
3 oz smoked salmon	227
3 oz beef liver	246
3 oz white tuna	535
3 oz salmon fillet	638

[2]U.S. Department of Agriculture, Agriculture Research Service, 1999. USDA Nutrient Database for Standard Reference, Release 13.

Omega-3 PUFAs induce apoptosis in cancer cells, whereas they protect neuronal, retinal, and cardiac cells against apoptosis. It is beyond the scope of this review to discuss the effect of ω-3s in every single health condition. Therefore, this review article will focus primarily on the role of ω-3 PUFAs in affecting signal transduction processes leading to apoptosis in various cancers.

Several reports have demonstrated that ω-3 PUFAs exert their anticancer effects on various cancer cell lines [44-47]. Dietary supplementation with ω-3 PUFAs (as a pure agent or in fish oil) increased apoptotic cell death in normal rat colonic cells [44-47], in transplantable rat Morris hepatocarcinoma 3924A cells [48], and in Walker 256 carcinosarcoma cells [49]. Omega-3 PUFAs suppress the progression of human breast MDA-MB-231 [50, 51], MDA-MB-435 [52, 53] and KPL-1 cancer cells [54] in athymic nude mice. They increase survival time for dogs with lymphoma [55] and reduce the risk of prostate cancer in humans [56]. Omega-3 PUFAs have also been shown to significantly reduce the incidence of tumor induction by

Table 4. Therapeutic Use of ω-3 PUFAs

Patent	Description	Inventor	Patent #
Glutamine-rich composition for immune system	For the purpose of treating patients whose immune systems have been weakened because of disease or trauma; the major ingredient is glutamine, which is accompanied by ω-3 and ω-6 PUFAs, arginine, and RNA.	Steven M. Ostrom	199861/10 USA
Formulation for menopausal women	For the purpose of providing nutritional supplementation for postmenopausal/menopausal women as well as relieving associated symptoms. The supplementation consists of various compounds including linoleic acid, linolenic acid, DHA, and other ω-3 fatty acids.	Saul R. Levinson *et al.*	131236/10 USA
Composition for increase in ω-3 of human cell membranes	Proposes to increase the amount of ω-3 fatty acids that comprise cell membranes by way of a parenteral injection of fatty acid triglycerides in the form of an isotonic lipid emulsion.	Yvon A. Carpentier Isabelle E. Dupont	01117991 USA
Nutritional formula for ICU patients	Provides a formula for administration to intensive care patients who have weakened states of absorption capacity. The formula is given enterally and consists of a source of protein, carbohydrate, and ω-3/ω-6 fatty acids.	John Alexander *et al.*	96202637 USA
Formulation for smokers	Formula for combating the negative effects of smoking, such as aging of the skin; consists of a combination of ω-3 and ω-6 fatty acids.	David F. Horrobin	94301853 USA
Pharmaceutical composition for morbid affections	A composition containing esters of ω-3 PUFAs that have proven to be clinically useful in the treatment of psoriasis and phlebitis.	Tiberio Bruzzese *et al.*	93110903 USA
Formulation for protective effect on the liver	A combination of ω-3 fatty acids in their esterified form accompanied by medium-length triglycerides. The triglycerides are preferentially oxidized and thus protect the fatty acids from rapid oxidation. This combination helps to protect the liver and suppresses chronic inflammatory disorders.	Dr. Jorg Nehne Michael Boll	88116623 USA
Emulsion containing ω-3 fatty acids for treating inflam-matory diseases	An emulsion that contains multiple ω-3 fatty acids or their esters along with traditional additives for the treatment of ulcerative colitis.	Jeffrey Askanazi *et al.*	00637957/EP B1
Drinkable ω-3 preparation	A liquid nutritional drink consisting of ω-3 PUFAs in a water-based solution that does not turn rancid with time	Johan Myhre AS Coromar	00147377 WO
Composition for maintenance of gut integrity	A method for restoring gut integrity by administering a combination of ω-3 and ω-6 PUFAs.	Susan Marie Kaup	00035443 WO

dimethylbenz(a)anthracene in rats [57]. Addition of ω-3 PUFAs to the cultures of lung carcinoma A427 cells [58], Hep2 human larynx tumor cells [59], pancreatic Mia-Pa-Ca-2 cells [60], and embryonal carcinoma Tera-2 cells [61] induces apoptosis in these cell lines. Omega-3 PUFAs also inhibit the growth of cervical cells immortalized by the highly oncogenic human papillomavirus 16 (HPV16), foreskin keratinocytes immortalized by HPV16, and keratinocytes grown from papillomas with an HPV etiology [62]. Furthermore, conjugated DHA with a triene structure has been shown to induce apoptosis in DLD-1 cells (colorectal adenocarcinoma) without any effect on normal human fibroblast cell lines [63]. While there are many examples of ω-3 PUFA-induced apoptosis, at present, the cellular and molecular mechanisms are unclear and a better understanding of the basic actions of ω-3 PUFAs will be needed before these polyunsaturated fatty acids can be fully employed in the clinic as anticancer agents [64, 65].

Many anticancer drugs exert their influence by inducing apoptosis. Apoptosis, or programmed cell death, is the physiological method by which unwanted or unneeded cells are eliminated during development or other biological processes [66]. It is also an important process in degenerative diseases, autoimmune disorders, and neoplasia development [67]. As a genetically regulated mechanism, apoptosis can occur through many pathways, but it is defined by several typical cellular and molecular events, including cell shrinkage, endoplasmic reticulum dilation, membrane blebbing, and extensive nuclear fragmentation [66]. Caspases, a family of cysteine proteases, play a critical role in apoptosis and are responsible for many of the biochemical and morphological changes associated with apoptosis [68-71].

OMEGA-3 FATTY ACIDS AND CYTOSOL-LINKED APOPTOSIS

Omega-3 PUFAs exert their anticancer effects by slowing down the growth of cancer cells *via* inhibition of cell cycle progression. However, in the continued presence of ω-3 PUFAs these arrested cells start dying through apoptosis. Previously, we demonstrated that DHA prolongs the S phase in cultured spleen lymphocytes [72]. Subsequently, other

Table 5. Functional Foods Containing ω-3 PUFA[3]

Product Type	Product	Manufacturer	ω-3 content/serving
Cereal	Healthy Scoop	Food by Design www.foodbydesign.com	2800 mg
Cereal	Cranberry Cereal Almonds Cereal Apple Cinnamon	Zoe Foods www.zoefoods.com	2400 mg
Cereal Bar	Healthy Break	Food by Design	2800 mg
Cereal Bar	Zoe Flax and Soy Bar -chocolate -peanut butter -apple crisp -lemon	Zoe Foods	1500 mg per bar for chocolate and peanut butter bars; 2200 mg per bar for apple crisp and lemon bars.
Cookies	Flax Macs	Food by Design	2400 mg
Eggs	Born 3 eggs	Born 3 Marketing Corp www.born3.com	400 mg
Mix	Flax Jacks (pancake and waffle mix)	Food by Design	5000 mg
Oil	Golden Omega-Omega oil	Naturalways www.naturalways.com/omega-omega	5750 mg

[3]Food websites found using www.flaxcouncil.ca/foodlist.

investigators demonstrated that ω-3 PUFAs arrest malignant cells in the S phase [73] and prevent G1/S progression in HT-29 human colonic cells [74], vascular smooth muscle cells [75], and urothelial cells [76]. These observations indicate that ω-3 PUFAs, particularly DHA, can exert their anticancer effects by arresting cell cycle progression. To date, however, little is known about the molecular and cellular events that lead to ω-3 PUFA-mediated cell cycle arrest and subsequent apoptosis. Progression through each phase of the cell cycle is tightly regulated and involves the expression and rapid degradation of the cyclin-dependent kinase (cdk) complex. In general, the levels of cdks are relatively constant throughout the cell cycle, while the cyclin levels vary substantially [77]. Cyclin A appears in the S phase with the onset of DNA synthesis [78]. Cyclin A associates initially with cyclin-dependent kinase-2 (cdk2) and later with cdk1 (also known as cell division control protein 2 or cdc2 and involved in G2/M progression) [78]. This association, and hence, the activities of cdk2 and cdc2, are essential for progression through the S phase to the G2 phase. Many of the effects of cyclin-cdks are mediated through phosphorylation of retinoblastoma protein (pRb) (Fig. (**3**)). pRb controls the progression of the cell cycle by regulating the activities of transcription factors, most importantly, E2F2 and E2F3. In a hypophosphorylated state, pRb physically associates with these transcriptional factors and blocks their ability to activate the gene expression of products necessary for cell cycle progression. Once phosphorylated, pRb loses much, if not all, of its growth inhibitory power and permits the advance into late G1, and hence, into the remainder of the cell cycle [79].

Table 6. Fatty Acid Composition of Selected ω–3 Supplements

	Monounsaturated	Omega-3 (mg/g oil)	Omega-6	w-3/w-6 ratios
Sundown Flax Oil	190 (150)	555 (530)	140 (129)	3.96
Sundown Cod Liver Oil	165 (???)	266 (160)	21.3 (???)	12.48
Sundown Fish Oil	388 (???)	243 (300)	20 (???)	12.15
Puritan's Pride	73 (???)	527(500)	28(???)	18.82
Nature Made	123 (???)	286 (360)	91 (45)	3.16
Member's Mark	138 (???)	323 (300)	30 (???)	10.7
Sigma Chem. Co.	160 (120-260)	313 (180-300)	43 (<60)	7.28

Number in parenthesis indicates the manufacturer's reported values on the label.

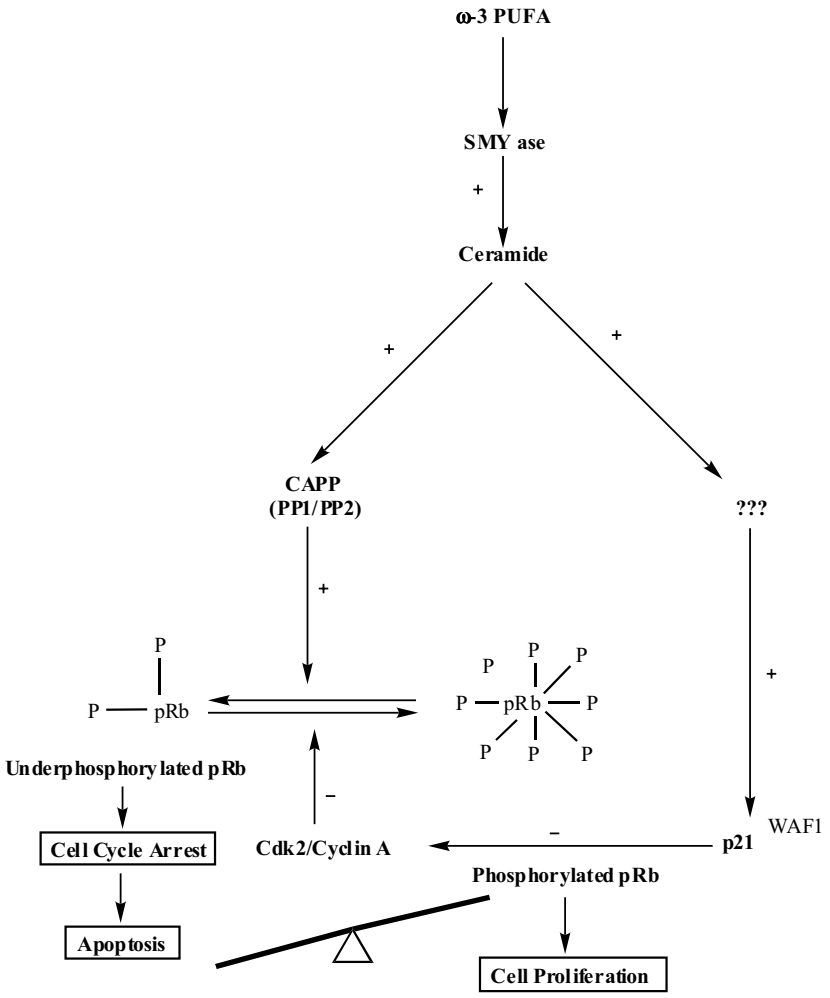

Fig. (3). Proposed action of ω-3 PUFAs on cell cycle arrest in cancer cells

Incorporation of ω-3 PUFAs into the cell membrane causes activation of sphingomyelinase (SMYase) and generation of ceramide. Ceramide mediates its effects *via* activation of ceramide-activated protein phosphatases (CAPP). Activation of CAPP (PP1 and/or PP2A) results in dephosphorylation of pRb phosphorylation. Ceramide also causes increased expression of $p21^{WAF1}$, and subsequently, inhibition of cyclin A/cdk2 activities. The overall effect of DHA results in hypophosphorylation of pRb protein and cell cycle arrest.

For the past 15 years our laboratory has investigated the relationship of DHA's alteration of membrane structure to its effects on cell signaling and apoptosis. Our initial studies used model lipid bilayers to explore the interaction of DHA with membrane phospholipids [80-85]. These experiments were then extended to Jurkat leukemia cells to elucidate the mechanism of the anticancer effects of DHA [86-88]. We demonstrated that low doses of DHA induce S phase cell cycle arrest in Jurkat cells through hypophosphorylation of pRb by inhibiting cdk2 kinase activity and stimulating protein phosphatase activities [88]. Our earlier studies using a model lipid bilayer suggest that DHA incorporation into membranes containing sphingomyelin and cholesterol affects the formation of a type of lipid micro domain known as "lipid rafts" [39]. Lipid rafts may also contain sphingomyelinase (SMYase), an enzyme that generates ceramide, a potent second messenger involved in cell cycle arrest and apoptosis [89, 90]. Ceramide levels also change during progression of the cell cycle [91]. We therefore examined the levels of ceramide in DHA-induced growth-arrested cells. As predicted, our data demonstrate that DHA causes increased ceramide formation [92], probably resulting from DHA-induced activation of SMYase in the plasma membranes. Ceramide is a known potent activator of a protein phosphatase specific for cdk2 [92] and also functions as a modulator of pRb phosphorylation [91]. Furthermore, our research has demonstrated that DHA treatment of Jurkat cells leads to the activation of protein phosphatase 1 (PP1) and 2A (PP2A) [86, 87]. Therefore, it appears that DHA-induced ceramide formation leads to activation of protein phosphatases and then, subsequent to these events, dephosphorylation of pRb. Our studies also indicate that DHA induces elevated levels of $p21^{WAF1}$. Ceramide has been shown to enhance expression of p21 [92], a cellular inhibitor of cdk2 kinase. Through the p21 mechanism, it is possible that elevated levels of ceramide lead to inhibition of cdk2 kinase. It therefore appears that DHA-induced ceramide may regulate phosphorylation of pRb by directly activating protein phosphatases and perhaps by inhibiting cyclin A/cdk2 activities *via* increased expression of $p21^{WAF1}$.

Although we have not yet studied the molecular mechanism by which ceramide leads to an increased expression of $p21^{WAF1}$, it is clear that PP1 is not involved upstream of $p21^{WAF1}$ expression. A role for ceramide in the induction of p21 *via* activation of nuclear factor kappa-B (NFκB) and/or p53 has been established by various studies [93, 94]. A possible mechanism for DHA-induced cell cycle arrest is outlined in Fig. (**3**).

Furthermore, we observed that growth-arrested cells undergo apoptosis upon repeated treatment with low doses of DHA. This apoptosis process appears to be mediated *via* caspase-3 activation [88]. Previously we suggested that activation of caspase-3, and hence, induction of apoptosis by DHA, is also mediated through activation of protein phosphatases [86]. Our studies are consistent with several others that have shown that apoptosis can be mediated by activation of protein phosphatases. For example, Wolf and Eastman [95] demonstrated that activation of PP1 plays an important role in Fas-induced apoptosis by stimulating mitochondrial release of cytochrome C and caspase activation in HL-60 and Jurkat cells. Similarly, activation of a PP2A-like phosphatase has been demonstrated to play a key role in inducing apoptosis in a neuronal cell line [96]. Other studies have shown that CAPP, a member of the PP2A family, is involved in receptor-mediated induction of apoptosis in various cell lines [97]. These studies suggest that protein phosphatase activation may be a common feature of cells undergoing apoptosis (Fig. (**4**)). However, at present it is not clear how DHA activates protein phosphatases and how activation of protein phosphatases is linked to cell cycle arrest and induction of apoptosis. We did not test the role of EPA in cell cycle arrest during this investigation. EPA can be converted to DHA, and therefore, it can have effects similar to DHA on cell cycle arrest. Indeed several investigators have demonstrated that EPA also blocks cell cycle progression and induces apoptosis in Ramos cells [98], squamous cell carcinoma cells [99], vascular smooth muscle cells [75], HT29 colonic cells [100], and pancreatic PaCa-2 cancer cells [101]. In most cases the results are consistent with our finding of growth arrest in the S phase of cell cycle progression [75, 100, 101] through inhibition of cdk2 activities [75].

While our studies probed one possible pathway for DHA's effect on cell growth and viability, many other, often overlapping possible modes of action undoubtedly exist.

OMEGA-3 FATTY ACIDS AND MITOCHONDRIA-LINKED APOPTOSIS

The involvement of mitochondria in apoptosis has been demonstrated by several investigators in recent years [102-107] and this pathway has also been strongly implicated in ω-3 PUFA-induced cell death. Omega-3 PUFAs have been reported to alter mitochondrial membrane properties and functions in rat colonocytes [108], the human colon tumor

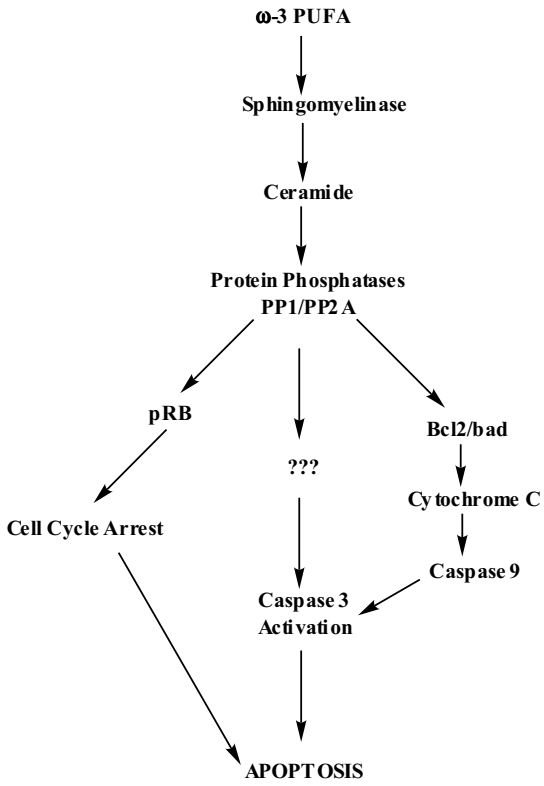

Fig. (4). Possible ω-3 PUFA-induced involvement of protein phosphatase in apoptosis. Activation of protein phosphates by ω-3 PUFA-induced ceramide formation can affect cancer cell growth through multiple pathways. Via dephosphorylation of retinoblastoma protein (pRB), protein phosphatases cause cell cycle arrest, which then leads to the induction of apoptosis. Protein phosphatases can also mediate release of cytochrome c and activation of caspases *via* dephosphorylation of Bcl2/Bid proteins. However, it is also possible that protein phosphatases play a direct role in the activation of caspase 3. Activation of the executionery caspase 3 then leads to the induction of apoptosis.

cell line HT29 [109], Walker 256 rat carcinosarcoma [49], T24 [49], and Hep2 [59] cancer cells.

Evidence suggests that DHA, but not EPA, preferentially accumulates in cardiolipin [109]. Cardiolipin (CL) is a diphospholipid (diphosphatidylglycerol) required for mitochondrial structural integrity and for the proper function of the electron transport chain [110]. CL is absent from all other cell membranes other than mitochondria, where it is present in the inner membrane and at intermembrane contact sites [110]. In tissues with high respiration rates, such as heart, CL can account for 25% of the phospholipids in the inner-mitochondrial membrane [111], where it is usually bound to the enzyme complexes of electron transport and ATP synthesis (i.e., cytochrome *c* oxidase [112-114], NADH reductase [115, 116], cytochrome b_1c_1 complex [116, 117], and ATP synthase [117, 118]). This suggests that mitochondrial function is very much dependent on the proper amount of CL. The CL acyl composition is sensitive to diet, and in humans it is usually rich in the essential dietary fatty acid linoleic acid (LA, 18:2 n-6) [119]. However, any change in dietary fatty acids is reflected in a change in acyl composition of CL. In mammals, CL has been modified to contain 85-90 mol% LA [120, 121], 50 mol% DHA [122], or 50 mol% oleic acid (OA) [122]. It is believed that ω-3 PUFAs in CL are susceptible to reactive oxygen species (ROS), which are generated through oxidative phosphorylation. CL is peroxidized by ROS and this process results in a decrease in CL levels in the mitochondrial membrane [123]. It has been suggested that low levels of CL either by peroxidation or its decreased synthesis [124, 125] compromises the integrity of CL-dependent proteins involved in energy metabolism, causing a drop in mitochondrial membrane potential, which in turn initiates apoptosis [126].

Consistent with these suggestions, cancer cells treated with ω-3 fatty acids clearly exhibit alterations in mitochondrial membrane potential and undergo apoptosis [49, 59, 108, 109]. Recently, a number of studies have reported a mechanism of mitochondrial-mediated apoptosis. It has been suggested that the peroxidation and/or loss of CL induces cytochrome C release from mitochondria. Studies have shown a highly significant temporal correlation of CL depletion with cytochrome C release to the cytosol [110]. The integrity of mitochondria and release of cytochrome C are regulated by Bcl-2 family members residing in the outer mitochondrial membrane [127]. Bcl-2 family members encode proteins that can be either antiapoptotic (e.g., Bcl-2, Bcl-X_L) or proapoptotic (e.g., Bax, Bcl-X_S, Bak, Bad, Bid) and therefore integrate signals from growth and death stimuli. An excess of Bcl-2 antiapoptotic proteins over Bcl-2 proapoptotic proteins protects the integrity of mitochondria and prevents cytochrome C release, whereas an excess of proapoptotic Bcl-2 proteins over antiapoptotic Bcl-2 proteins allows leakage of cytochrome C. It has been demonstrated recently that one chain of CL is inserted into a hydrophobic channel in cytochrome C, whereas another acyl chain extends into the bilayer [128]. CL is released from mitochondria for degradation in peroxisomes [129]. The proapoptotic protein Bid plays a role in the transfer of CL [130]. It has been shown that CL transfer occurs at the same concentrations of Bid that lead to mitochondrial release of cytochrome C [130]. The activities of the Bcl-2 family are regulated by different mechanisms, such as homo- and heterodimerization with other family members and also by proteolysis and phosphorylation [131]. There are recent reports that antiapoptotic Bcl-2 is inactivated by phosphatases, particularly by PP2A [132]. We have demonstrated that DHA treatment of Jurkat leukemia cells results in ceramide formation [88], which is also known to activate a phosphatase with characterized PP2A-type properties [97]. Similarly, dephosphorylation of Bad (proapoptotic) results in its activation and binding to Bcl-2, initiating cytochrome C release. The release of cytochrome C then interacts with Apaf-1 and dATP, leading to caspase 9 activation and hence downstream execution of the caspase cascade [133, 134]. The effector caspases are active proteases that then lead to morphological changes characteristic of apoptotic cell death, such as membrane blebbing and formation of apoptotic vesicles, cytoplasmic shrinkage, nuclear condensation, and DNA fragmentation. The omega-3-induced steps in mitochondria-linked apoptosis are outlined in Fig. (**4**).

SUMMARY

Epidemiological and dietary studies strongly indicate that ω-3 PUFAs provide tremendous health benefits for a number of diseases, including cancer and heart disease. ω-3 PUFAs are readily obtained from naturally occurring foods, manufactured functional foods, or from ω-3 PUFA supplements. One has to be careful, however, as our study suggests that different brands of supplements contain widely different amounts of ω-3 PUFAs and other mono- and polyunsaturated fatty acids and cholesterol. ω-3 PUFAs have been shown to alter biologically essential processes, including cell signaling and apoptosis. Here we reviewed how ω-3 PUFAs might regulate cellular signaling pathways and *induce* apoptosis through cytosolic and mitochondrial mediated signaling pathways. But even this is controversial, as ω-3 PUFAs have been shown to *prevent* apoptosis in heart, neuronal, and retinal tissues. In these organs, ω-3 PUFAs appear to preserve function and exhibit antiapoptotic properties through similar cellular signaling pathways that induce apoptosis in other organs. Comparing details of the effects of ω-3 PUFAs on cell signaling in different tissues therefore offers a unique approach in developing ω-3 PUFA-containing drugs. These drugs may selectively destroy cancer cells while preserving the vital physiological functions of other healthy tissues.

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