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The polyunsaturated fatty acids from microalgae as potential sources for health and disease

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Abstract: In the classification of the plant kingdom, the microalgae occupy a particular place from their geographical localization, their behavior with respect to the environmental constraints but also from their morphology, their physiology and their biochemistry. These various aspects make their cellular machineries very flexible and able to produce many organic molecules with various biological activities such as proteins, polysaccharides, pigments and lipids of interest. With regard to the lipids, microalgae are able to produce many varieties and great amounts of fatty acids, especially of polyunsaturated fatty acids, making them candidates of interest within the framework of the production and exploitation of lipids in various sectors of industry and health. These fatty acids present in the lipids produced by microalgae can be used alike manner as those resulting from marine sources of animal origin such as fish in the field from health. The aims of this chapter are to present the biochemical diversity of the lipids and the fatty acids contained in various species of microalgae of marine and freshwater environments. Then, this review will give examples of how these plant resources can represent an alternative to the animal ones, particularly fish, in the area of the prevention of pathologies such as cardiovascular diseases and cancers.

1. Introduction

Microalgae are photosynthetic unicellular organisms belonging to phytoplankton and, as primary producers of marine and freshwater ecosystems, have large potential not only as feeding other organisms in the food chain but also as producer of several metabolites with high added value [1]. Among the total volume of water of the Earth, more than 95% are represented by marine water (oceans representing 70% of the area of the planet) while only 3% was constituted by freshwater. There are over 50,000 different species of microalgae, found in benthic and littoral habitats, of which only a few have been characterized [2,3]. They include commonly organisms such as diatoms, dinoflagellates, green and yellow-brown flagellates and, sometimes, up to cyanobacteria. Most of the microalgae leave in suspension in the water column and movement can be detected during season variations, through blooms of algae and microalgae on area of the seas. The group of microalgae is diverse in morphology and in its biochemical composition, including pigments, lipids, fatty acids, vitamins, sterols and polysaccharides [4-6]. Therefore microalgae have a great potential in several economic fields such as aquatic nutrition and biodiesel production [5] and several culture conditions have been developed including laboratory scale conditions to produce microalgae with controlled parameters or outdoor production, for the industrial use. Actually, it is estimated that numerous active substances originating from marine organisms are used in the industry and can be used as an alternative source of chemically synthesized medicine in health, cosmetics, pharmaceuticals and new fuels [5,7-9]. Beside their mediatic use as a source of biodiesel, microalgae also appears as an alternative source of lipids and especially polyunsaturated fatty acids (PUFAs) of interest for human health.

2. Microalgae as an alternative source of lipids

Because the common source for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), *i.e.* fish oil, fails to meet the increasing demand for purified EPA and DHA, alternative sources such as microalgae that may contain large quantities of high-quality EPA and DHA are considered as potential source of these economically-important fatty acids, especially under heterotrophic growth conditions that could reduced the costs of lipids production [10-12]. Indeed, numerous works have described microalgae as producers of various lipids, including neutral and polar lipids such as glycerides, phospholipids and galactolipids, and fatty acids having biological activities in health field and especially PUFAs of the *n-3* series [5,7,13-21].

The lipid content of algae, as expressed related to the microalga dry weight, is considered to be in a range of 1 to more than 70% according environmental conditions [22]. Lipids contain glycerol, oses, or bases esterified to fatty acids. Lipid analyses established that microalgae contain saturated, monounsaturated and polyunsaturated fatty acids (Figure 1). Among the fatty acids, the *n-3* and *n-6* series have interest [23]. However, numerous microalgae are known to contain various fatty acids. The main constituents of the lipid fraction of *Chlorella vulgaris* are oleic (18:1*n-9*), palmitic (16:0) and linolenic (18:3*n-3*) acids, accounting for 41, 22 and 9% of the total amount, respectively [24]. In *Dunaliella salina* these fatty acids account for more than 80% of the total of fatty acids [25]. The diversity of fatty acids that can be produce by microalgae is also in function of length or unsaturation index. For example, Rodríguez-Meizoso et al. established the short chain fatty acid composition of the green microalga *Haematococcus* [26].

Actually, fish and fish oil are the main sources of long chain PUFAs (*n-3*) but it has been raised pollutants or toxins could be accumulated in fish. Moreover, the use of fish oil is quite poor partially due to problems linked with odor, taste and oxidative stability [27]. Consequently, production of long chain *n-3* PUFAs by microalgae is in increasing development for incorporation into infant milk formulations and for use as dietary supplements and food additives [27]. Under optimal culture conditions, *Chlorella minutissima* can produce an EPA content of up to 45% of its total fatty acid content [20]. Microalga such as *Porphyridium* sp., which shows a relatively low lipid content, contains significant amounts of several major fatty acids such as palmitic acid, ARA (20:4*n-6*), EPA (20:5*n-3*) and linoleic (18:2*n-6*) acid [28,29]. *Spirulina* provides an interesting source of γ -linolenic (18:3*n-6*) acid (20–25% of the total lipid fraction), a precursor of prostaglandins, leukotrienes and thromboxans involved in the modulation of immunological, inflammatory and cardiovascular responses [30]. This microalga is also a natural source of active fatty acids such as lauric (12:0), palmitic and oleic acids, with the *n-3* fatty acid DHA (22:6*n-3*) accounting for up to 9.1% of the total fatty acids content [31]. Used as dietary supplement, microalgae rich in EPA or in DHA, *Odontella aurita* and *Isochrysis galbana*, respectively, have been reported to have beneficial effects in health [17,32].

Microalgae are known to produce and accumulate *n-6* PUFA. One of the most studied microalga is the green freshwater *Parietochloris incisa* because is the richest plant source of arachidonic acid (ARA). In this microalga, ARA is incorporated into the triacylglycerols (TAG) while usually, saturated or monounsaturated fatty acids resides in these lipids. This microalga has the ability to transfer ARA from TAG to polar lipids according to

environmental temperatures. Indeed, from 25°C to 12 or 4°C, ARA is incorporated into the PC and DGTS polar lipids [33]. This ability can be interesting according the bioavailability of fatty acid depending on lipid in which they are incorporated, for human nutrition

Beneficial effects of marine food ingredients and dietary supplements are generally due to the *n*-3 PUFAs, specifically EPA and DHA. As described above microalgae contain not only *n*-3 PUFA but also unsaturated or *n*-6 fatty acids. In the following sections, benefits ascribed to the *n*-3 PUFAs will be described in the context of cardiovascular diseases and cancer.

3. Microalgae PUFAs and health benefits

Microalgae represent a major resource of valuable bioactive compounds and biochemicals such as pigments, polysaccharides, sterols, polyunsaturated fatty acids and vitamins with potential applications in the food, pharmaceutical and cosmetics industries [4-6,34]. Therefore, the health benefits of microalgae are being investigated and more recognized and appreciated within the last three to four decades, especially since the introduction of probiotic nutritional supplements [35]. Beneficial health effects of food ingredients and dietary supplements are attributed to a great extent to long chain polyunsaturated fatty acids (LC-PUFAs). These LC-PUFAs cannot be synthesized by higher plants and animals and only healthy human adults are able to elongate 18:3*n*-3 to EPA in an extend lower than 5% and convert EPA to DHA in a rate inferior to 0.05%, being inhibited in childhood and elderly life [36,37]. However, some microalgae have the ability to synthesize LC-PUFAs with particular interest (Figure 4), namely γ -linolenic acid (GLA, 18:3*n*-6, *Arthrospira*), ARA (20:4*n*-6, *Porphyridium*), EPA (20:5*n*-3, *Nannochloropsis*, *Phaeodactylum*, *Nitzschia*, *Isochrysis*, *Diacronema*) and DHA(22:6*n*-3, *Cryptocodinium*, *Schizochytrium*) [14,19,27,38,39].

LC-PUFAs, especially of *n*-3 and *n*-6 series such as EPA, DHA, and ARA are considered pharmacologically important for dietetics and therapeutics [34] and have been used for prophylactic and therapeutic treatment of chronic inflammations (rheumatism, skin diseases, and inflammation of the mucosa of the gastrointestinal tract). Also, they are believed to have a positive effect on cardiovascular diseases, coronary heart diseases, atherosclerosis, hypertension, cholesterol, and cancer treatment [35].

3.1. Cardioprotective effects of *n*-3 PUFAs

There are some epidemiologic data indicating that populations with a high intake of *n*-3 PUFAs, such as Eskimos and Japanese in fishing villages, have a low risk of cardiovascular diseases [40,41]. There is convincing evidence that marine products (fish or microalgae) rich

in *n-3* LC-PUFA (especially in EPA, 20:5 *n-3* and DHA, 22:6 *n-3*) reduce the risk of cardiovascular diseases (CVD) and a dietary deficiency in *n-3* LC-PUFA is firmly linked to increased morbidity and mortality from coronary heart disease.

In the literature, recommended daily intakes of *n-3* LC-PUFA vary from 200 mg to 1 g EPA and DHA [42]. Recent evidence shows that the intake of EPA and DHA is inversely related to cardiovascular risk in a dose-dependent manner up to about 250 mg/day in healthy populations, and intake of 1 g/day is associated with a marked protection from sudden cardiac death [43-46].

In fact, *n-3* PUFAs have the ability to reduce the risk of cardiovascular disease, through a reduction of factors involved in metabolic syndrome development, by modification of serum lipid profile. The main effect of *n-3* PUFAs on plasma lipids is a reduction of the concentration of plasma triacylglycerols. EPA and DHA are mainly involved in a reduction of TAG synthesis and adiposity. During obesity, the body mass index and waist circumference are inversely correlated with EPA and DHA intakes [47]. These effects are attributable to the increased lipolysis and decreased lipogenesis, mainly in the liver, that have a central role in the control of whole-body lipid homeostasis [48]. In addition, there are several reports indicating that consumption of *n-3* PUFAs increases the levels of plasma HDL-cholesterol in human [49-51]. Hypotensive effects of *n-3* PUFAs have been shown in animal and clinical studies, and seem to be correlated to the plasma phospholipids composition in EPA and DHA that contribute to modulation of membrane fluidity, activities of membrane enzymes and receptors, and production of eicosanoids [52]. *n-3* PUFAs consumption has been associated with the regulation of eicosanoid production which are bioactive substances that influence various functions in cells and tissues being important in the prophylaxis and therapy of chronic and degenerative diseases including reduction of blood cholesterol, protection against cardiovascular, coronary heart diseases, atherosclerosis, diabetes, hypertension and metabolic diseases [53-56]. Other important role of *n-3* PUFAs is attributed to gene expression regulation, as well as cholesterol and fasting triacylglycerol (TAG) decreases [57].

The cardioprotective effects of the *n-3* PUFAs has been reported through many epidemiological studies in human and animal models but also in cell culture studies [58-61]. It has been shown that *n-3* fatty acids improve disorders related with obesity increasing the fatty acid beta-oxidation and the adiponectin levels in serum of obese rats [62].

In type 2 diabetes patients, dietary EPA and DHA have been shown to reduce TAG and VLDL-cholesterol levels and to increase the HDL-cholesterol level, in blood [63]. Patients with dyslipidemia have been shown to have significant decrease in blood TAG and increase in

HDL-cholesterol after a consumption of EPA and DHA during 3 and 6 months [64]. Adan et al. showed that EPA and DHA feeding reduces serum cholesterol and TAG levels, and decreases platelet aggregation in hypercholesterolemic rats [65].

Recent studies in hypercholesterolemic rats have shown that DHA supplementation reduced total weight gain, adiposity index, HDL-cholesterol and glucose plasmatic concentration regardless of the dose and form of supplementation [66]. In addition EPA and DHA also play a key role in normalizing platelet hyper-aggregability. When added to the diet, EPA and DHA can alter the phospholipid membrane composition of the cells, and impact on the synthesis and action of eicosanoids, and regulate transcription factor activity and abundance [67-70].

Microalgae LC-PUFAs and cardiovascular diseases (CVD)

Many studies have reported high amounts of *n*-3 fatty acids such as EPA and DHA in fish and other marine sources such as microalgae [5,20,21]. Fish oil supplements and other sources such as microalgae provide EPA and DHA usually used for human diets [17,71]. For example, the marine diatom *Odontella aurita* is one of the microalgae known to be rich in EPA and currently approved as a dietary supplement [6,17]. During dyslipidemia in rats, the use of the marine diatom *Odontella aurita* has shown beneficial effects as a reduction in the risk factors for high-fat induced metabolic syndrome such as hyperlipidemia, platelet aggregation and oxidative stress [17]. Due to its ability to produce PUFAs, mainly DHA and EPA, the microalga *Isochrysis galbana* [72] induced a decreased glucose, triacylglycerol and cholesterol blood levels in alloxan-induced diabetic rats. However, an increased light-density lipoprotein level and a decreased high-density lipoprotein level were observed in the diabetic rats, but also in the healthy ones [32].

Some studies showed beneficial effects of *Chlorella*, especially when administered on the presence of underlying disorders such as in streptozotocin induced diabetes rats [73,74]. Cherng et al. also showed that *Chlorella pyrenoidosa* has the ability to prevent dyslipidemia in rats and hamsters models fed chronic high fat and could be potential in use to prevent intestinal absorption of redundant lipid from daily intake and subsequently to prevent hyperlipidemia as well as atherosclerosis [75]. Feeding animals with *Chlorella pyrenoidosa* enhances the hypoglycemic effects of exogenous insulin at a dose, which does not produce hypoglycemia in streptozotocin-induced diabetic mice, suggesting that insulin sensitivity is increased in these mice [73,74]. In rabbit fed a high cholesterol diet for 10 weeks, *Chlorella vulgaris*, another species of *Chlorella*, showed anti-lipidemic and anti-atherosclerotic actions

[71]. Another observation has indicated that *Chlorella* intake can reduce cholesterol levels in patients with hypercholesterolemia [76].

Arthrospira sp. (*Spirulina* sp.) grows profusely in certain alkaline lakes in Mexico and Africa and has been used for ages as food by local populations [77]. It is extensively produced around the world (3000 tons/year) and broadly used in food and feed supplements, due of its high protein content and its excellent nutritive value, such as high γ -linolenic acid level (20–25% of the total lipid fraction) [78,79], which is a precursor of prostaglandins, leukotrienes and thromboxans involved in the modulation of immunological, inflammatory and cardiovascular responses [30]. In addition, this microalga has various possible health promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, suppression of elevated serum glucose level [27], anticarcinogenic effects and have hypocholesterolemic properties [80].

The dinoflagellate *Cryptothecodinium cohnii* seems the most efficient microorganism for the large-scale production of DHA devoid of EPA. The marine protists Thraustochytrids offer promising possibilities for DHA and other major PUFA production. *Cryptothecodinium cohnii* as well as *Thraustochytrium* and *Schizochytrium* are able to produce large biomass and lipid amounts, and DHA at levels up to 60%. Now, organically produced DHA-rich microalgae oil is available. Clinical trials with DHA-rich oil indicate comparable efficacies to fish oil for protection from cardiovascular risk factors by lowering plasma triglycerides and oxidative stress [81,82].

3.2. Cancer preventive effect of *n*-3 PUFAs

Although increased fat consumption has been associated with the development of specific types of cancer such as breast, colonic and pancreatic cancer, epidemiological studies have shown that the rate of breast cancer is 4 to 5 times higher in Western countries than in Japan suggesting that diet, and particularly diet rich in *n*-3 and *n*-6 long-chain polyunsaturated fatty acids (PUFAs), may have an influence on tumor emergence (reviewed in [69,83,84]). Saturated fatty acids and mono-unsaturated fatty acids have been shown to have only a weak effect on promoting tumors whereas *n*-6 PUFA have been associated with a greater capacity to induce tumor formation [83,85]. By contrast, *n*-3 PUFAs are thought to have a cancer preventive action and a high dietary intake of fish is associated with a lower incidence of cancers [86-88]. However cohort studies that examined the effect of *n*-3 PUFAs on breast, colorectal and prostate cancer incidence yielded mixed results, and most of them did not show a significant association between *n*-3 PUFAs consumption and cancer risk [89-91].

Nevertheless the Women's Intervention Nutrition Study (WINS) provided evidence that a reduction in dietary fat intake to 22% of total energy intake led to a 24% reduction in the recurrence rate of breast cancer [92]. Similarly, a recent meta-analysis of data from 489 000 individuals showed insufficient evidence of a protective effect of *n-3* fatty acids on colorectal cancer risk [93]. However, a reduced risk observed in men warrants further investigations [93]. Besides, about 20 clinical studies have investigated the use of *n-3* PUFAs from fish oil or purified EPA and DHA in the treatment or prevention of cancer cachexia suggesting the interest of long chain PUFAs as adjuvant of conventional cancer therapy [84,94-97].

Marine microalgae have been identified as an important alternative source of DHA and EPA and could be used to replace fish oil which may be depleted in the future by the overharvesting of *n-3* PUFA rich fish [21,98]. Indeed algal oils rich in DHA are nutritionally equivalent to fish oils in several tests [98] and extracts from the marine EPA-rich microalgae *Odontella aurita* demonstrated anti-proliferative effect on cultures of bronchopulmonary and epithelial cells [99]. The mechanism by which DHA and EPA could provide protection against the appearance of a tumor, or directly influence cancer cells by reducing their malignancy, remains unclear, since cohort studies do not reveal any correlation between fat intake and cancer [89,100]. Nevertheless, some evidence hints that DHA not only acts as an anti-proliferative agent by lengthening the cell cycle between the G2/M transition [101], but is also a proapoptotic factor, increasing caspase-3 and the Bax protein level [102,103]. In addition, DHA has been shown to affect cell proliferation, whatever its source (i.e., fish oil or microalgae) [104]. It has also been shown that the *n-3* PUFAs and DHA, in particular, can act on lipid peroxidation as well as on the proteins implicated in the ROS mechanism leading to cell death [105,106]. It is also possible that DHA can induce several different pathways leading to apoptosis, and in particular, the Bax pathway that has been described for the HL60 cell line [103]. Besides, other authors have shown that DHA has a cytotoxic effect on cancer cells by decreasing the level of superoxide dismutase 1, allowing an increase in lipid peroxidation to occur [107]. This suggests that DHA could reduce tumor numbers by acting as soon as a cell begins to change and becomes pre-cancerous; this might explain the low level of breast cancer in populations with a high DHA diet. Furthermore, one of the main problems in most of cancer is the ability of cancer cells to metastasize and some studies have shown that diet can affect the metastatic potential of cancer cell lines known to have a high metastatic phenotype (reviewed in [69]).

Several studies showed that DHA and EPA together or alone inhibit the growth of cancer cell lines [108-112]. In addition, DHA was shown to act both as an antiproliferative agent by lengthening the cell cycle between the G2/M transition [101], and as a proapoptotic factor (Figure 2), increasing Bcl-2, procaspase-8, and caspase-3 activity in cancer cell lines [70,102,113]. Incorporation of *n-3* PUFAs in membranes decreased arachidonic acid (AA) content and *n-6/n-3* PUFA ratio in the membranes, without modifying the unsaturation index [114]. Consequently, the modification of AA metabolism, especially the inhibition of the production of eicosanoids, may explain in part the antiproliferative and proapoptotic effect of *n-3* PUFAs [115,116]. Associated with growth arrest and apoptosis, an increased lipid peroxidation and ROS production was reported in *n-3* PUFA-treated cancer cells [105,106,117-122] suggesting a role for ROS in mediating the antiproliferative effect of *n-3* PUFAs. Indeed, the reduced form of glutathione (GSH) as well as cytosolic glutathione peroxidase activity were decreased in cancer cells treated with DHA [106,119,121] whereas main antioxidant enzyme activities (i.e., superoxide dismutase and catalase) were increased [106,119]. However, some differences between DHA and EPA may be noted as reported in glioblastoma cells where the levels of reactive oxygen species and thiobarbituric acid-reactive substances were significantly higher in DHA-treated cells than in EPA- and AA-treated groups [119]. Finally, the use of various antioxidant molecules was shown to inhibit *n-3* PUFA-induced apoptosis, suggesting the involvement of lipid peroxidation-derived ROS [105,106,117-122].

The *n-3* PUFAs may also exert their growth inhibitory effects on cancer cells by altering the plasma membrane composition and associated signaling events [68]. One emerging view is that DHA-containing phospholipids modify the biophysical organization of the plasma membrane which in turn modifies protein activity and cellular functions [123-127]. Model membrane studies suggest that the energetically less favorable interaction between cholesterol and PUFA, especially DHA, promotes lateral phase segregation into sterol-poor/PUFA-rich and sterol-rich/saturated fatty acid-rich microdomains [126,128,129]. Since lipid rafts are predominantly enriched in saturated fatty acids containing sphingolipids and cholesterol, the incorporation of PUFA, especially DHA, determines in cancer cells a disruption of lipid rafts and a formation of the PUFA-rich/cholesterol-poor non-raft domains [125]. The effect of *n-3* PUFAs on lipid rafts and their signaling pathways has been studied in several cell types with cancerous origin or not [130]. Particularly, the raft marker caveolin-1 is partially displaced on treatment with DHA and EPA [131,132]. In the MDA-MB-231 breast cancer cell line, Altenburg and Siddiqui showed that *n-3* PUFAs exposure resulted in a decreased level of the

chemokine receptor CXCR4, which requires intact lipid rafts for signaling [133]. Schley et al. have shown in the MDA-MB-231 cell line that a combination of EPA and DHA induces a modification in the lipid raft composition including fatty acids, phospholipids, cholesterol, ceramides, and DAG content of membrane rafts [134]. These alterations of lipid content are accompanied by a decrease of EGFR in rafts and an increased whole cells level of phosphorylated EGFR and p38 MAPK [134]. Increased phosphorylation of EGFR and p38 was already reported as a proapoptotic signal in cancer cells [134-138]. Furthermore DHA induces the upregulation of EGFR tyrosine phosphorylation and the increase of EGFR association with the Sos1 guanine nucleotide protein exchange factor in cancer cell lines including MDA-MB-231 [139]. These data suggest that EGF/Ras/Erk signaling is being disrupted in DHA-treated breast cancer cells by the exclusion of EGFR protein from lipid raft microdomains [134,139]. Corsetto et al. have recently examined the PUFA incorporation in breast cancer lipid rafts and showed that PUFA are incorporated preferentially in phosphatidylinositol, phosphatidylserine, and phosphatidylcholine that may be relevant to the formation of biologically active metabolites such as prostaglandins, prostacyclins, leukotrienes, resolvines, and protectines [125]. These authors conclude that while EPA may contribute to cell apoptosis mainly through a decrease of AA concentration in lipid raft phospholipids, DHA may change the biophysical properties of lipid rafts decreasing the content of cholesterol and the distribution of key proteins such as EGFR, Src, heterotrimeric G-proteins subunits, or sphingomyelinase. Indeed DHA decreases the sphingomyelin content in lipid rafts of breast cancer cell lines [125]. This might be due to an activation of sphingomyelinase, leading to the production of ceramide, which is well known to be associated with apoptosis and cellular stress [140-143]. Increased activity of the neutral sphingomyelinase in response to EPA and DHA treatment was previously reported in Jurkat leukemic cells [116] and *n*-3 PUFA-mediated alteration of lipid rafts was linked to oxidative stress modulation [123]. In human endothelial cells, DHA reduced oxidative-stress-induced calcium influx through modification of lipid raft composition [127]. Then PUFAs appeared as proliferation inhibitors and apoptosis inducers in cancers at least in part through remodeling of lipid rafts, MAPK and ROS pathways [69].

PUFA-Induced Inhibition of Cell Migration and Invasiveness

Because metastasis is the leading cause of death from cancer, reducing the invasive potential of cancer cells is almost as important as destroying in the primary tumor. Recently, we showed that DHA reduces the invasive potential of the MDA-MB-231 breast cancer cell line

(Figure 3) [113]. Interestingly cholesterol levels in lipid rafts, which are altered by *n-3* PUFA [134], are critical for the migration, invasion, and angiogenesis of cancer cells [144]. As an example, methyl- β -cyclodextrin reduced uPAR and matrix metalloproteinase-9 (MMP-9) colocalization in lipid rafts and inhibited breast carcinoma cell migration and invasion [144]. In addition the DHA induced reduction of breast cancer cells migration may also be due to inhibition of voltage-gated Na⁺ channels [145,146]. Indeed DHA inhibits voltage-gated Na⁺ channels (neonatal Nav1.5) in a dose-dependent manner, and tetrodotoxin, a compound that specifically blocks this type of channels, reduces MDA-MB-231 cell migration at the same level that observed in the presence of DHA [146]. The authors concluded that DHA-induced suppression of cellular migration occurred primarily via down-regulation of voltage-gated Na⁺ channel mRNA and functional protein expression [146]. Moreover voltage-gated Na⁺ channels localization in lipid rafts, such as shown in cardiac cells [147], may be affected by PUFAs [145]. As another example, exposure of MDA-MB-231 breast cancer cells to *n-3* PUFAs results in decreased surface levels of the main chemokine receptor CXCR4, and in a reduction of the CXCR4 ligand-dependant migration of cells [133]. This suggests that the disruption of required lipid raft domains for CXCR4 signaling and the displacement of CXCR4 from the lipid raft domains are potential mechanisms behind the inhibited migratory response after DHA and EPA treatment [133].

4. Conclusion

The use of microalga in our lifespan will continue to be more and more attractive as many molecules derived from these microorganisms can be beneficial to our health and particularly in the metabolic disease and cancers [5,6]. Microalgae can be considered as the future main providers of PUFAs and other metabolites having high added values [5]. A selective modulation of the production of these molecules by microalgae might be possible. In this way it is of interest to increase our knowledge about the control of carbon metabolism in these organisms and their growing parameters such as the water temperature, pH, salinity, light and nutrients under a stress condition [148,149]. For example it is known that astaxanthin is preferentially produced by *Haematococcus pluvialis* under light stress [148]. Consequently, this brings to biotechnological perspectives to produce nutraceuticals compounds in both food and pharmaceutical industries. Food industry will be able to produce these nutraceuticals for new food processes in order to fight against obesity and big pharma will be able to use the microalga potential as well to research new natural molecules with a better target against some cancers that can improve lower side effects on patients. Indeed the potential of DHA has

already been shown as an adjuvant in chemotherapy [150]. Finally the relative amounts of *n*-6 PUFA to *n*-3 PUFAs may be more important for cardiovascular disease and cancer risk than individual dietary amounts of these fatty acids, and PUFAs may also have interesting synergistic effect with canonical treatments that make them considered as powerful nontoxic adjuvants [84,94-97].

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References

- [1] El Gamal, A.A. (2010). Biological importance of marine algae. *Saudi Pharm. J.* 18, 1-25.
- [2] Bhakuni, D.S. and Rawat, D.S. (2005) Bioactive marine natural products (Bhakuni, D.S. and Rawat, D.S., eds), pp. 396. Anamaya Publishers, New Delhi, India.
- [3] Guiry, M.D. (2012). How many species of algae are there? *J. Phycol.* 48, 1057-1063.
- [4] Mata, T.M., Martins, A.A. and Caetano, N.S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* 14, 217-232.
- [5] Mimouni, V., Ulmann, L., Pasquet, V., Mathieu, M., Picot, L., Bougaran, G., Cadoret, J.P., Morant-Manceau, A. and Schoefs, B. (2012). The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. *Curr. Pharm. Biotechnol.* 13, 2733-2750.
- [6] Heydarizadeh, P., Poirier, I., Loizeau, D., Ulmann, L., Mimouni, V., Schoefs, B. and Bertrand, M. (2013). Plastids of marine phytoplankton produce bioactive pigments and lipids. *Mar. Drugs*, in press.
- [7] Imhoff, J.F., Labes, A. and Wiese, J. (2011). Bio-mining the microbial treasures of the ocean: New natural products. *Biotechnol. Adv.* 29, 468-482.
- [8] Sharma, K.K., Schuhmann, H. and Schenk, P.M. (2012). High lipid induction in microalgae for biodiesel production. *Energies* 5, 1532-1553.
- [9] Thomas, N.V. and Kim, S.K. (2013). Beneficial effects of marine algal compounds in cosmeceuticals. *Mar. Drugs* 11, 146-164.
- [10] Chi, Z., Pyle, D., Wen, Z., Frear, C. and Chen, S. (2007). A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgal fermentation. *Process Biochem.* 42, 1537-1545.
- [11] Sijtsma, L. and de Swaaf, M.E. (2004). Biotechnological production and applications of the omega-3 polyunsaturated fatty acid docosahexaenoic acid. *Appl. Microbiol. Biotechnol.* 64, 146-153.
- [12] Wen, Z.Y. and Chen, F. (2003). Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotechnol. Adv.* 21, 273-294.
- [13] Guihéneuf, F., Mimouni, V., Ulmann, L. and Tremblin, G. (2009). Combined effects of irradiance level and carbon source on fatty acid and lipid class composition in the

- microalga *Pavlova lutheri* commonly used in mariculture. *J. Exp. Mar. Biol. Ecol.* 369, 136-143.
- [14] Bandarra, N.M., Pereira, P.A., Batista, I. and Vilela, M.H. (2003). Fatty acids, sterols and α -tocopherol in *Isochrysis galbana*. *J. Food Lipids* 10, 35-43.
- [15] Bigogno, C., Khozin-Goldberg, I., Boussiba, S., Vonshak, A. and Cohen, Z. (2002). Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid. *Phytochem.* 60, 497-503.
- [16] Chen, G.Q., Jiang, Y. and Chen, F. (2007). Fatty acid and lipid class composition of the eicosapentaenoic acid-producing microalga, *Nitzschia laevis*. *Food Chem.* 104, 1580-1585.
- [17] Haimeur, A., Ulmann, L., Mimouni, V., Gueno, F., Pineau-Vincent, F., Meskini, N. and Tremblin, G. (2012). The role of *Odontella aurita*, a marine diatom rich in EPA, as a dietary supplement in dyslipidemia, platelet function and oxidative stress in high-fat fed rats. *Lipids Health Dis.* 11, 147.
- [18] Krienitz, L. and Wirth, M. (2006). The high content of polyunsaturated fatty acids in nannochloropsis limnetica (eustigmatophyceae) and its implication for food web interactions, freshwater aquaculture and biotechnology. *Limnol. Ecol. Manag. Inland Waters* 36, 204-210.
- [19] Molina Grima, E., Belarbi, E.H., Acien Fernandez, F.G., Robles Medina, A. and Chisti, Y. (2003). Recovery of microalgal biomass and metabolites: Process options and economics. *Biotechnol. Adv.* 20, 491-515.
- [20] Rasmussen, R.S. and Morrissey, M.T. (2007). Marine biotechnology for production of food ingredients. *Adv. Food Nutr. Res.* 52, 237-292.
- [21] Ward, O.P. and Singh, A. (2005). Omega-3/6 fatty acids: Alternative source of production. *Process Biochem.* 40, 3627-3652.
- [22] Metting, F.B.J. (1996). Biodiversity and application of microalgae. *J. Industrial Microbiol.* 17, 477-489.
- [23] Brown, M.R., Jeffrey, S.W., Volkman, J.K. and Dunstan, G.A. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture* 151, 315-331.
- [24] Mendes, R.L., Fernandes, H.L., Coelho, J., Reis, E.C., Cabral, J.M.S., Novais, J.M. and Palavra, A.F. (1995). Supercritical CO₂ extraction of carotenoids and other lipids from *Chlorella vulgaris*. *Food Chem.* 53, 99-103.
- [25] Herrero, M., Ibanez, E., Cifuentes, A., Reglero, G. and Santoyo, S. (2006). *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. *J. Food Prot.* 69, 2471-2477.
- [26] Rodríguez-Meizoso, I., Jaime, L., Santoyo, S., Señoráns, F.J., Cifuentes, A. and Ibáñez, E. (2010). Subcritical water extraction and characterization of bioactive compounds from *Haematococcus pluvialis* microalga. *J. Pharm. Biomed. Analysis* 51, 456-463.
- [27] Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006). Commercial applications of microalgae. *J. Biosci. Bioeng.* 101, 87-96.
- [28] Plaza, M., Herrero, M., Cifuentes, A. and Ibanez, E. (2009). Innovative natural functional ingredients from microalgae. *J. Agric. Food Chem.* 57, 7159-7170.
- [29] Reboloso Fuentes, M. (2000). Biomass nutrient profiles of the microalga *Porphyridium cruentum*. *Food Chem.* 70, 345-353.
- [30] Burtin, P. (2003). Nutritional value of seaweeds. *Electron. J. Environ. Agric. Food Chem.* 2, 498-503.
- [31] Mendiola, J.A., Jaime, L., Santoyo, S., Reglero, G., Cifuentes, A., Ibáñez, E. and Señoráns, F.J. (2007). Screening of functional compounds in supercritical fluid extracts from *Spirulina platensis*. *Food Chem.* 102, 1357-1367.

- [32] Nuño, K., Villarruel-López, A., Puebla-Pérez, A.M., Romero-Velarde, E., Puebla-Mora, A.G. and Ascencio, F. (2013). Effects of the marine microalgae *Isochrysis galbana* and *Nannochloropsis oculata* in diabetic rats. *J. Function. Foods* 5, 106-115.
- [33] Bigogno, C., Khozin-Goldberg, I. and Cohen, Z. (2002). Accumulation of arachidonic acid-rich triacylglycerols in the microalga *Parietochloris incisa* (Trebuxiophyceae, Chlorophyta). *Phytochem.* 60, 135-143.
- [34] Pulz, O. and Gross, W. (2004). Valuable products from biotechnology of microalgae. *Appl. Microbiol. Biotechnol.* 65, 635-648.
- [35] Barrow, C. and Shahidi, F. (2007) Marine nutraceuticals and functional foods. CRC Press, Taylor & Francis Ed.
- [36] Burdge, G.C. and Calder, P.C. (2005). Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* 45, 581-597.
- [37] Wang, C., Harris, W.S., Chung, M., Lichtenstein, A.H., Balk, E.M., Kupelnick, B., Jordan, H.S. and Lau, J. (2006). N-3 fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: A systematic review. *Am. J. Clin. Nutr.* 84, 5-17.
- [38] Chini Zitelli, G., Tomasello, V., Pinzani, E. and Tredici, M.R. (1996). Outdoor cultivation of *Arthrospira platensis* during autumn and winter in temperate climates. *J. Applied Phycol.* 8, 293-301.
- [39] Donato, M., Vilela, M.H. and Bandarra, N.M. (2003). Fatty acids, sterols, alpha-tocopherol and total carotenoids composition of *Diacronema vlkianum*. *J. Food Lipids* 10, 267-276.
- [40] Kagawa, Y., Nishizawa, M., Suzuki, M., Miyatake, T., Hamamoto, T., Goto, K., Motonaga, E., Izumikawa, H., Hirata, H. and Ebihara, A. (1982). Eicosapolyenoic acids of serum lipids of japanese islanders with low incidence of cardiovascular diseases. *J. Nutr. Sci. Vitaminol. (Tokyo)* 28, 441-453.
- [41] Newman, W.P., Middaugh, J.P., Propst, M.T. and Rogers, D.R. (1993). Atherosclerosis in alaska natives and non-natives. *Lancet* 341, 1056-1057.
- [42] Kris-Etherton, P.M. and Hill, A.M. (2008). N-3 fatty acids: Food or supplements? *J. Am. Diet Assoc.* 108, 1125-1130.
- [43] GISSI Prevenzione Investigators. (1999). Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: Results of the gissi-prevenzione trial. Gruppo italiano per lo studio della sopravvivenza nell'infarto miocardico. *Lancet* 354, 447-455.
- [44] Mozaffarian, D. (2008). Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. *Am. J. Clin. Nutr.* 87, 1991S-1996S.
- [45] Rupp, H., Wagner, D., Rupp, T., Schulte, L.M. and Maisch, B. (2004). Risk stratification by the "EPA+DHA level" and the "EPA/AA ratio" focus on anti-inflammatory and antiarrhythmogenic effects of long-chain omega-3 fatty acids. *Herz* 29, 673-685.
- [46] European Food Safety Authority. (2009). Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *European Food Safety Authority Journal* 1176, 1-11.
- [47] Micallef, M., Munro, I., Phang, M. and Garg, M. (2009). Plasma n-3 polyunsaturated fatty acids are negatively associated with obesity. *Br. J. Nutr.* 102, 1370-1374.
- [48] Harris, W.S. and Bulchandani, D. (2006). Why do omega-3 fatty acids lower serum triglycerides? *Curr. Opin. Lipidol.* 17, 387-393.
- [49] Kesavulu, M.M., Kameswararao, B., Apparao, C., Kumar, E.G. and Harinarayan, C.V. (2002). Effect of omega-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients. *Diabetes Metab.* 28, 20-26.

- [50] Mori, T.A. and Beilin, L.J. (2004). Omega-3 fatty acids and inflammation. *Curr. Atheroscler. Rep.* 6, 461-467.
- [51] Sirtori, C.R., Crepaldi, G., Manzato, E., Mancini, M., Rivellesse, A., Paoletti, R., Pazzucconi, F., Pamparana, F. and Stragliotto, E. (1998). One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: Reduced triglyceridemia, total cholesterol and increased HDL-C without glycaemic alterations. *Atherosclerosis* 137, 419-427.
- [52] Kinsella, J.E., Lokesh, B. and Stone, R.A. (1990). Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: Possible mechanisms. *Am. J. Clin. Nutr.* 52, 1-28.
- [53] Bonna, K.H., Bjerve, K.S., Straume, B., Gram, I.T. and Thelle, D. (1990). Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the tromso study. *New Engl. J. Med.* 322, 795-801.
- [54] Sidhu, K.S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. *Regul. Toxicol. Pharmacol.* 38, 336-344.
- [55] Simopoulos, A.P. (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.* 21, 495-505.
- [56] Thies, F., Garry, J.M., Yaqoob, P., Rerkasem, K., Williams, J., Shearman, C.P., Gallagher, P.J., Calder, P.C. and Grimble, R.F. (2003). Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: A randomised controlled trial. *Lancet* 361, 477-485.
- [57] Calder, P.C. (2004). N-3 fatty acids and cardiovascular disease: Evidence explained and mechanisms explored. *Clin. Sci. (Lond.)* 107, 1-11.
- [58] Duda, M.K., O'Shea, K.M. and Stanley, W.C. (2009). Omega-3 polyunsaturated fatty acid supplementation for the treatment of heart failure: Mechanisms and clinical potential. *Cardiovasc. Res.* 84, 33-41.
- [59] Lee, J.H., O'Keefe, J.H., Lavie, C.J. and Harris, W.S. (2009). Omega-3 fatty acids: Cardiovascular benefits, sources and sustainability. *Nat. Rev. Cardiol.* 6, 753-758.
- [60] Saremi, A. and Arora, R. (2009). The utility of omega-3 fatty acids in cardiovascular disease. *Am. J. Ther.* 16, 421-436.
- [61] Yashodhara, B.M., Umakanth, S., Pappachan, J.M., Bhat, S.K., Kamath, R. and Choo, B.H. (2009). Omega-3 fatty acids: A comprehensive review of their role in health and disease. *Postgrad. Med. J.* 85, 84-90.
- [62] Shirouchi, B., Nagao, K., Inoue, N., Ohkubo, T., Hibino, H. and Yanagita, T. (2007). Effect of dietary omega 3 phosphatidylcholine on obesity-related disorders in obese Otsuka long-Evans Tokushima fatty rats. *J. Agric. Food Chem.* 55, 7170-7176.
- [63] Valdivielso, P., Rioja, J., Garcia-Arias, C., Sanchez-Chaparro, M.A. and Gonzalez-Santos, P. (2009). Omega 3 fatty acids induce a marked reduction of apolipoprotein B48 when added to fluvastatin in patients with type 2 diabetes and mixed hyperlipidemia: A preliminary report. *Cardiovasc. Diabetol.* 8, 1.
- [64] Derosa, G., Maffioli, P., D'Angelo, A., Salvadeo, S.A., Ferrari, I., Fogari, E., Gravina, A., Mereu, R., Randazzo, S. and Cicero, A.F. (2009). Effects of long chain omega-3 fatty acids on metalloproteinases and their inhibitors in combined dyslipidemia patients. *Expert Opin. Pharmacother.* 10, 1239-1247.
- [65] Adan, Y., Shibata, K., Sato, M., Ikeda, I. and Imaizumi, K. (1999). Effects of docosahexaenoic and eicosapentaenoic acid on lipid metabolism, eicosanoid production, platelet aggregation and atherosclerosis in hypercholesterolemic rats. *Biosci. Biotechnol. Biochem.* 63, 111-119.

- [66] Tabernero, M., Bermejo, L.M., Elvira, M., Caz, V., Baeza, F., Senorans, F.J., De Miguel, E. and Largo, C. (2013). Metabolic effect of docosahexaenoic acid supplementation in different doses and formulations (ethyl- and glyceryl-) in hypercholesterolemic rats. *J. Function. Foods* 5, 755-762.
- [67] Benatti, P., Peluso, G., Nicolai, R. and Calvani, M. (2004). Polyunsaturated fatty acids: Biochemical, nutritional and epigenetic properties. *J. Am. Coll. Nutr.* 23, 281-302.
- [68] Chapkin, R.S., McMurray, D.N., Davidson, L.A., Patil, B.S., Fan, Y.Y. and Lupton, J.R. (2008). Bioactive dietary long-chain fatty acids: Emerging mechanisms of action. *Br. J. Nutr.* 100, 1152-1157.
- [69] Chénais, B. and Blanckaert, V. (2012). The janus face of lipids in human breast cancer: How polyunsaturated fatty acids affect tumor cell hallmarks. *Int. J. Breast Cancer* 2012, 712536.
- [70] Corsetto, P.A., Montorfano, G., Zava, S., Jovenitti, I.E., Cremona, A., Berra, B. and Rizzo, A.M. (2011). Effects of n-3 PUFAs on breast cancer cells through their incorporation in plasma membrane. *Lipids Health Dis.* 10, 73.
- [71] Lee, S.H., Kang, H.J., Lee, H.J., Kang, M.H. and Park, Y.K. (2010). Six-week supplementation with *Chlorella* has favorable impact on antioxidant status in Korean male smokers. *Nutrition* 26, 175-183.
- [72] Liu, C.P. and Lin, L.P. (2001). Ultrastructural study and lipid formation of *Isochrysis* sp. Ccmp 1324. *Bot. Bull. Acad. Sinica* 42, 207-214.
- [73] Cherng, J.Y. and Shih, M.F. (2005). Potential hypoglycemic effects of *Chlorella* in streptozotocin-induced diabetic mice. *Life Sci.* 77, 980-990.
- [74] Cherng, J.Y. and Shih, M.F. (2006). Improving glycogenesis in streptozocin (STZ) diabetic mice after administration of green algae *Chlorella*. *Life Sci.* 78, 1181-1186.
- [75] Cherng, J.Y. and Shih, M.F. (2005). Preventing dyslipidemia by *Chlorella pyrenoidosa* in rats and hamsters after chronic high fat diet treatment. *Life Sci* 76, 3001-3013.
- [76] Okudo, M., Hasegawa, T., Sonoda, M., Okabe, T. and Tanaka, M. (1975). The effects of *Chlorella* on the level of cholesterol in serum and liver. *Jpn. J. Nutr.* 33, 3-8.
- [77] Yamaguchi, K. (1997). Recent advances in microalgal bioscience in japan, with special reference to utilization of biomass and metabolites: A review. *J. Applied Phycol.* 8, 227-233.
- [78] Otles, S. and Pire, R. (2001). Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. *J. AOAC Int.* 84, 1708-1714.
- [79] Shimamatsu, H. (2004). Mass production of *Spirulina*, an edible microalga. *Hydrobiologia* 512, 39-44.
- [80] Reinehr, C.O. and Costa, J.A.V. (2006). Repeated batch cultivation of the microalga *Spirulina* *platensis*. *World J. Microbiol. Biotechnol.* 22, 937-943.
- [81] Erkkila, A.T., Matthan, N.R., Herrington, D.M. and Lichtenstein, A.H. (2006). Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD. *J. Lipid Res.* 47, 2814-2819.
- [82] Stark, K.D. and Holub, B.J. (2004). Differential eicosapentaenoic acid elevations and altered cardiovascular disease risk factor responses after supplementation with docosahexaenoic acid in postmenopausal women receiving and not receiving hormone replacement therapy. *Am. J. Clin. Nutr.* 79, 765-773.
- [83] de Lorgeril, M. and Salen, P. (2012). New insights into the health effects of dietary saturated and omega-6 and omega-3 polyunsaturated fatty acids. *BMC Med.* 10, 50.
- [84] Vaughan, V.C., Hassing, M.R. and Lewandowski, P.A. (2013). Marine polyunsaturated fatty acids and cancer therapy. *Br. J. Cancer* 108, 486-492.

- [85] Weisburger, J.H. (1997). Dietary fat and risk of chronic disease: Mechanistic insights from experimental studies. *J. Am. Diet Assoc.* 97, S16-23.
- [86] Hursting, S.D., Thornquist, M. and Henderson, M.M. (1990). Types of dietary fat and the incidence of cancer at five sites. *Prev. Med.* 19, 242-253.
- [87] Kaizer, L., Boyd, N.F., Kriukov, V. and Tritchler, D. (1989). Fish consumption and breast cancer risk: An ecological study. *Nutr. Cancer* 12, 61-68.
- [88] Sasaki, S., Horacsek, M. and Kesteloot, H. (1993). An ecological study of the relationship between dietary fat intake and breast cancer mortality. *Prev. Med.* 22, 187-202.
- [89] Gerber, L.H., Stout, N.L., Schmitz, K.H. and Stricker, C.T. (2012). Integrating a prospective surveillance model for rehabilitation into breast cancer survivorship care. *Cancer* 118, 2201-2206.
- [90] Holmes, M.D., Liu, S., Hankinson, S.E., Colditz, G.A., Hunter, D.J. and Willett, W.C. (2004). Dietary carbohydrates, fiber, and breast cancer risk. *Am. J. Epidemiol.* 159, 732-739.
- [91] MacLean, C.H., Newberry, S.J., Mojica, W.A., Khanna, P., Issa, A.M., Suttorp, M.J., Lim, Y.W., Traina, S.B., Hilton, L., Garland, R. and Morton, S.C. (2006). Effects of omega-3 fatty acids on cancer risk: A systematic review. *JAMA* 295, 403-415.
- [92] Chlebowski, R.T., Blackburn, G.L., Thomson, C.A., Nixon, D.W., Shapiro, A., Hoy, M.K., Goodman, M.T., Giuliano, A.E., Karanja, N., McAndrew, P., Hudis, C., Butler, J., Merkel, D., Kristal, A., Caan, B., Michaelson, R., Vinciguerra, V., Del Prete, S., Winkler, M., Hall, R., Simon, M., Winters, B.L. and Elashoff, R.M. (2006). Dietary fat reduction and breast cancer outcome: Interim efficacy results from the women's intervention nutrition study. *J. Natl. Cancer Inst.* 98, 1767-1776.
- [93] Shen, X.J., Zhou, J.D., Dong, J.Y., Ding, W.Q. and Wu, J.C. (2012). Dietary intake of n-3 fatty acids and colorectal cancer risk: A meta-analysis of data from 489 000 individuals. *Br. J. Nutr.* 108, 1550-1556.
- [94] Cai, F., Dupertuis, Y.M. and Pichard, C. (2012). Role of polyunsaturated fatty acids and lipid peroxidation on colorectal cancer risk and treatments. *Curr. Opin. Clin. Nutr. Metab. Care* 15, 99-106.
- [95] Murphy, R.A., Mourtzakis, M. and Mazurak, V.C. (2012). N-3 polyunsaturated fatty acids: The potential role for supplementation in cancer. *Curr. Opin. Clin. Nutr. Metab. Care* 15, 246-251.
- [96] Siddiqui, R.A., Harvey, K.A., Xu, Z., Bammerlin, E.M., Walker, C. and Altenburg, J.D. (2011). Docosahexaenoic acid: A natural powerful adjuvant that improves efficacy for anticancer treatment with no adverse effects. *Biofactors* 37, 399-412.
- [97] Wang, J., Luo, T., Li, S. and Zhao, J. (2012). The powerful applications of polyunsaturated fatty acids in improving the therapeutic efficacy of anticancer drugs. *Expert Opin. Drug Deliv.* 9, 1-7.
- [98] van Beelen, V.A., Spenkeliink, B., Mooibroek, H., Sijtsma, L., Bosch, D., Rietjens, I.M. and Alink, G.M. (2009). An n-3 PUFA-rich microalgal oil diet protects to a similar extent as a fish oil-rich diet against aom-induced colonic aberrant crypt foci in f344 rats. *Food Chem. Toxicol.* 47, 316-320.
- [99] Moreau, D., Tomasoni, C., Jacquot, C., Kaas, R., Le Guedes, R., Cadoret, J.P., Muller-Feuga, A., Kontiza, I., Vagias, C., Roussis, V. and Roussakis, C. (2006). Cultivated microalgae and the carotenoid fucoxanthin from *Odontella aurita* as potent anti-proliferative agents in bronchopulmonary and epithelial cell lines. *Environ. Toxicol. Pharmacol.* 22, 97-103.
- [100] Holmes, M.D. and Willett, W.C. (2004). Does diet affect breast cancer risk? *Breast Cancer Res.* 6, 170-178.

- [101] Barascu, A., Besson, P., Le Floch, O., Bougnoux, P. and Jourdan, M.L. (2006). Cdk1-cyclin B1 mediates the inhibition of proliferation induced by omega-3 fatty acids in MDA-MB-231 breast cancer cells. *Int. J. Biochem. Cell. Biol.* 38, 196-208.
- [102] Chamras, H., Ardashian, A., Heber, D. and Glaspy, J.A. (2002). Fatty acid modulation of MCF-7 human breast cancer cell proliferation, apoptosis and differentiation. *J. Nutr. Biochem.* 13, 711-716.
- [103] Miura, Y., Takahara, K., Murata, Y., Utsumi, K., Tada, M. and Takahata, K. (2004). Docosahexaenoic acid induces apoptosis via the bax-independent pathway in HL-60 cells. *Biosci. Biotechnol. Biochem.* 68, 2415-2417.
- [104] Jude, S., Roger, S., Martel, E., Besson, P., Richard, S., Bougnoux, P., Champeroux, P. and Le Guennec, J.Y. (2006). Dietary long-chain omega-3 fatty acids of marine origin: A comparison of their protective effects on coronary heart disease and breast cancers. *Prog. Biophys. Mol. Biol.* 90, 299-325.
- [105] Siddiqui, R.A., Harvey, K. and Stillwell, W. (2008). Anticancer properties of oxidation products of docosahexaenoic acid. *Chem. Phys. Lipids* 153, 47-56.
- [106] Vibet, S., Goupille, C., Bougnoux, P., Steghens, J.P., Gore, J. and Maheo, K. (2008). Sensitization by docosahexaenoic acid (DHA) of breast cancer cells to anthracyclines through loss of glutathione peroxidase (GPX1) response. *Free Radic. Biol. Med.* 44, 1483-1491.
- [107] Ding, W.Q., Vaught, J.L., Yamauchi, H. and Lind, S.E. (2004). Differential sensitivity of cancer cells to docosahexaenoic acid-induced cytotoxicity: The potential importance of down-regulation of superoxide dismutase 1 expression. *Mol. Cancer Ther.* 3, 1109-1117.
- [108] Chajes, V., Sattler, W., Stranzl, A. and Kostner, G.M. (1995). Influence of n-3 fatty acids on the growth of human breast cancer cells in vitro: Relationship to peroxides and vitamin-E. *Breast Cancer Res. Treat.* 34, 199-212.
- [109] Grammatikos, S.I., Subbaiah, P.V., Victor, T.A. and Miller, W.M. (1994). N-3 and n-6 fatty acid processing and growth effects in neoplastic and non-cancerous human mammary epithelial cell lines. *Br. J. Cancer* 70, 219-227.
- [110] Schley, P.D., Jijon, H.B., Robinson, L.E. and Field, C.J. (2005). Mechanisms of omega-3 fatty acid-induced growth inhibition in MDA-MB-231 human breast cancer cells. *Breast Cancer Res. Treat.* 92, 187-195.
- [111] Davidson, J., Rotondo, D., Rizzo, M.T. and Leaver, H.A. (2012). Therapeutic implications of disorders of cell death signalling: Membranes, micro-environment, and eicosanoid and docosanoid metabolism. *Br. J. Pharmacol.* 166, 1193-1210.
- [112] Das, U.N. (1991). Tumoricidal action of cis-unsaturated fatty acids and their relationship to free radicals and lipid peroxidation. *Cancer Lett.* 56, 235-243.
- [113] Blanckaert, V., Ulmann, L., Mimouni, V., Antol, J., Brancquart, L. and Chénais, B. (2010). Docosahexaenoic acid intake decreases proliferation, increases apoptosis and decreases the invasive potential of the human breast carcinoma cell line MDA-MB-231. *Int. J. Oncol.* 36, 737-742.
- [114] Calviello, G., Palozza, P., Franceschelli, P. and Bartoli, G.M. (1997). Low-dose eicosapentaenoic or docosahexaenoic acid administration modifies fatty acid composition and does not affect susceptibility to oxidative stress in rat erythrocytes and tissues. *Lipids* 32, 1075-1083.
- [115] Hammamieh, R., Sumaida, D., Zhang, X., Das, R. and Jett, M. (2007). Control of the growth of human breast cancer cells in culture by manipulation of arachidonate metabolism. *BMC Cancer* 7, 138.
- [116] Wu, M., Harvey, K.A., Ruzmetov, N., Welch, Z.R., Sech, L., Jackson, K., Stillwell, W., Zaloga, G.P. and Siddiqui, R.A. (2005). Omega-3 polyunsaturated fatty acids

- attenuate breast cancer growth through activation of a neutral sphingomyelinase-mediated pathway. *Int. J. Cancer* 117, 340-348.
- [117] Kang, K.S., Wang, P., Yamabe, N., Fukui, M., Jay, T. and Zhu, B.T. (2010). Docosahexaenoic acid induces apoptosis in MCF-7 cells in vitro and in vivo via reactive oxygen species formation and caspase 8 activation. *PLoS One* 5, e10296.
- [118] Kikawa, K.D., Herrick, J.S., Tateo, R.E., Mouradian, M., Tay, J.S. and Pardini, R.S. (2010). Induced oxidative stress and cell death in the A549 lung adenocarcinoma cell line by ionizing radiation is enhanced by supplementation with docosahexaenoic acid. *Nutr. Cancer* 62, 1017-1024.
- [119] Leonardi, F., Attorri, L., Di Benedetto, R., Di Biase, A., Sanchez, M., Nardini, M. and Salvati, S. (2005). Effect of arachidonic, eicosapentaenoic and docosahexaenoic acids on the oxidative status of C6 glioma cells. *Free Radic. Res.* 39, 865-874.
- [120] Lindskog, M., Gleissman, H., Ponthan, F., Castro, J., Kogner, P. and Johnsen, J.I. (2006). Neuroblastoma cell death in response to docosahexaenoic acid: Sensitization to chemotherapy and arsenic-induced oxidative stress. *Int. J. Cancer* 118, 2584-2593.
- [121] Merendino, N., Loppi, B., D'Aquino, M., Molinari, R., Pessina, G., Romano, C. and Velotti, F. (2005). Docosahexaenoic acid induces apoptosis in the human PACA-44 pancreatic cancer cell line by active reduced glutathione extrusion and lipid peroxidation. *Nutr. Cancer* 52, 225-233.
- [122] Saw, C.L., Huang, Y. and Kong, A.N. (2010). Synergistic anti-inflammatory effects of low doses of curcumin in combination with polyunsaturated fatty acids: Docosahexaenoic acid or eicosapentaenoic acid. *Biochem. Pharmacol.* 79, 421-430.
- [123] Aliche-Djoudi, F., Podechard, N., Chevanne, M., Nourissat, P., Catheline, D., Legrand, P., Dimanche-Boitrel, M.T., Lagadic-Gossman, D. and Sergent, O. (2011). Physical and chemical modulation of lipid rafts by a dietary n-3 polyunsaturated fatty acid increases ethanol-induced oxidative stress. *Free Radic. Biol. Med.* 51, 2018-2030.
- [124] Calder, P.C. (2011). Fatty acids and inflammation: The cutting edge between food and pharma. *Eur. J. Pharmacol.* 668 Suppl 1, S50-58.
- [125] Corsetto, P.A., Cremona, A., Montorfano, G., Jovenitti, I.E., Orsini, F., Arosio, P. and Rizzo, A.M. (2012). Chemical-physical changes in cell membrane microdomains of breast cancer cells after omega-3 PUFA incorporation. *Cell Biochem. Biophys.* 64, 45-59.
- [126] Shaikh, S.R., Locascio, D.S., Soni, S.P., Wassall, S.R. and Stillwell, W. (2009). Oleic- and docosahexaenoic acid-containing phosphatidylethanolamines differentially phase separate from sphingomyelin. *Biochim. Biophys. Acta* 1788, 2421-2426.
- [127] Ye, S., Tan, L., Ma, J., Shi, Q. and Li, J. (2010). Polyunsaturated docosahexaenoic acid suppresses oxidative stress induced endothelial cell calcium influx by altering lipid composition in membrane caveolar rafts. *Prostaglandins Leukot. Essent. Fatty Acids* 83, 37-43.
- [128] Wassall, S.R. and Stillwell, W. (2008). Docosahexaenoic acid domains: The ultimate non-raft membrane domain. *Chem. Phys. Lipids* 153, 57-63.
- [129] Wassall, S.R. and Stillwell, W. (2009). Polyunsaturated fatty acid-cholesterol interactions: Domain formation in membranes. *Biochim. Biophys. Acta* 1788, 24-32.
- [130] Siddiqui, R.A., Harvey, K.A., Zaloga, G.P. and Stillwell, W. (2007). Modulation of lipid rafts by omega-3 fatty acids in inflammation and cancer: Implications for use of lipids during nutrition support. *Nutr. Clin. Pract.* 22, 74-88.
- [131] Li, Q., Zhang, Q., Wang, M., Liu, F., Zhao, S., Ma, J., Luo, N., Li, N., Li, Y., Xu, G. and Li, J. (2007). Docosahexaenoic acid affects endothelial nitric oxide synthase in caveolae. *Arch. Biochem. Biophys.* 466, 250-259.

- [132] Li, Q., Zhang, Q., Wang, M., Zhao, S., Xu, G. and Li, J. (2008). N-3 polyunsaturated fatty acids prevent disruption of epithelial barrier function induced by proinflammatory cytokines. *Mol. Immunol.* 45, 1356-1365.
- [133] Altenburg, J.D. and Siddiqui, R.A. (2009). Omega-3 polyunsaturated fatty acids down-modulate CXCR4 expression and function in MDA-MB-231 breast cancer cells. *Mol. Cancer Res.* 7, 1013-1020.
- [134] Schley, P.D., Brindley, D.N. and Field, C.J. (2007). (n-3) PUFA alter raft lipid composition and decrease epidermal growth factor receptor levels in lipid rafts of human breast cancer cells. *J. Nutr.* 137, 548-553.
- [135] Im, E. and Martinez, J.D. (2004). Ursodeoxycholic acid (UDCA) can inhibit deoxycholic acid (DCA)-induced apoptosis via modulation of EGFR/Raf-1/Erk signaling in human colon cancer cells. *J. Nutr.* 134, 483-486.
- [136] Qiu, L., Zhou, C., Sun, Y., Di, W., Scheffler, E., Healey, S., Wanebo, H., Kouttab, N., Chu, W. and Wan, Y. (2006). Paclitaxel and ceramide synergistically induce cell death with transient activation of EGFR and ERK pathway in pancreatic cancer cells. *Oncol. Rep.* 16, 907-913.
- [137] Squires, M.S., Hudson, E.A., Howells, L., Sale, S., Houghton, C.E., Jones, J.L., Fox, L.H., Dickens, M., Prigent, S.A. and Manson, M.M. (2003). Relevance of mitogen activated protein kinase (MAPK) and phosphatidylinositol-3-kinase/protein kinase b (PI3K/PKB) pathways to induction of apoptosis by curcumin in breast cells. *Biochem. Pharmacol.* 65, 361-376.
- [138] Cuadrado, A., Garcia-Fernandez, L.F., Gonzalez, L., Suarez, Y., Losada, A., Alcaide, V., Martinez, T., Fernandez-Sousa, J.M., Sanchez-Puelles, J.M. and Munoz, A. (2003). Aplidin induces apoptosis in human cancer cells via glutathione depletion and sustained activation of the epidermal growth factor receptor, Src, JNK, and p38 MAPK. *J. Biol. Chem.* 278, 241-250.
- [139] Rogers, K.R., Kikawa, K.D., Mouradian, M., Hernandez, K., McKinnon, K.M., Ahwah, S.M. and Pardini, R.S. (2010). Docosahexaenoic acid alters epidermal growth factor receptor-related signaling by disrupting its lipid raft association. *Carcinogenesis* 31, 1523-1530.
- [140] Dimanche-Boitrel, M.T., Rebillard, A. and Gulbins, E. (2011). Ceramide in chemotherapy of tumors. *Recent Pat. Anticancer Drug Discov.* 6, 284-293.
- [141] Furuya, H., Shimizu, Y. and Kawamori, T. (2011). Sphingolipids in cancer. *Cancer Metastasis Rev.* 30, 567-576.
- [142] Lafont, E., Kitatani, K., Okazaki, T. and Segui, B. (2011). Regulation of death and growth signals at the plasma membrane by sphingomyelin synthesis: Implications for hematological malignancies. *Recent Pat. Anticancer Drug Discov.* 6, 324-333.
- [143] Nikolova-Karakashian, M.N. and Rozenova, K.A. (2010). Ceramide in stress response. *Adv. Exp. Med. Biol.* 688, 86-108.
- [144] Raghu, H., Sodadasu, P.K., Malla, R.R., Gondi, C.S., Estes, N. and Rao, J.S. (2010). Localization of UPAR and MMP-9 in lipid rafts is critical for migration, invasion and angiogenesis in human breast cancer cells. *BMC Cancer* 10, 647.
- [145] Gillet, L., Roger, S., Bougnoux, P., Le Guennec, J.Y. and Besson, P. (2011). Beneficial effects of omega-3 long-chain fatty acids in breast cancer and cardiovascular diseases: Voltage-gated sodium channels as a common feature? *Biochimie* 93, 4-6.
- [146] Isbilen, B., Fraser, S.P. and Djamgoz, M.B. (2006). Docosahexaenoic acid (omega-3) blocks voltage-gated sodium channel activity and migration of MDA-MB-231 human breast cancer cells. *Int. J. Biochem. Cell. Biol.* 38, 2173-2182.

- [147] Maguy, A., Hebert, T.E. and Nattel, S. (2006). Involvement of lipid rafts and caveolae in cardiac ion channel function. *Cardiovasc Res.* 69, 798-807.
- [148] Lemoine, Y. and Schoefs, B. (2010). Secondary ketocarotenoid astaxanthin biosynthesis in algae: A multifunctional response to stress. *Photosynthesis Res.* 106, 155-177.
- [149] Heydarizadeh, P., Marchand, J., Chénais, B., Sabzalian, M.R., Zahedi, M., Moreau, B. and Schoefs, B. (2013). Functional investigation in diatoms needs more than a transcriptomic approach. *Diatom Res.*, in press
- [150] Bougnoux, P., Hajjaji, N., Ferrasson, M.N., Giraudeau, B., Couet, C. and Le Floch, O. (2009). Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: A phase II trial. *Br. J. Cancer* 101, 1978-1985.

Figure Legends

Figure 1. Marine microalga *n*-3 fatty acids and major polar lipids. A, α -linolenic acid (ALA, 18:4n-3), octadeca-9z,12z,15z-trienoic acid; B, eicosapentaenoic acid (EPA, 20:5n-3), eicosa-5z,8z,11z,14z,17z-pentaenoic acid; C, docosahexaenoic acid (DHA, 22:6n-3), docosa-4z,7z,10z,13z,16z,19z-hexaenoic acid; D, monogalactosyldiacylglycerol (MGDG); E, digalactosyldiacylglycerol (DGDG); F, sulfoquinovosyldiacylglycerol (SQDG); G, phosphatidylglycerol (PG).

Figure 2. DHA-induced apoptosis in the MDA-MB-231 breast cancer cell line. Nuclear staining by Hoechst method of: A, control cells with normal nuclei; B, C and D, 72h-treated cells (100 μ M DHA) showing apoptotic nuclei with condensed nuclear bodies (arrows). Bars correspond to 25 μ m.

Figure 3. Reduction of the invasive potential of breast cancer cells by DHA. The MDA-MB-231 cell line was treated with 100 μ M DHA for 24h incubation then invasive cells were counted using Boyden chamber's assay with Matrigel[®].

Figure 1

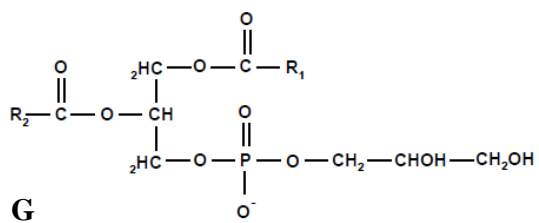
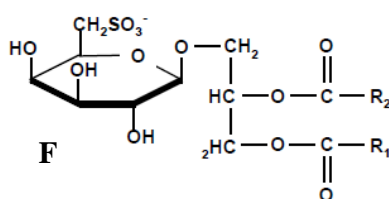
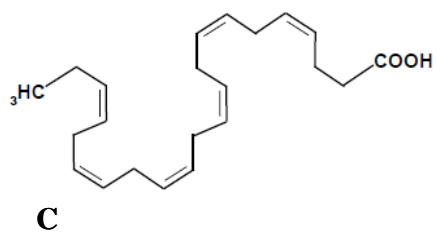
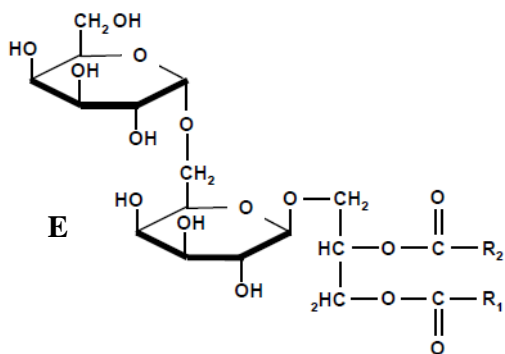
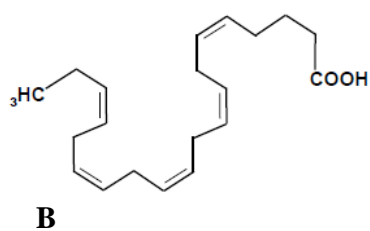
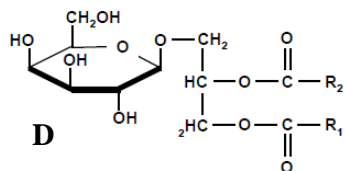
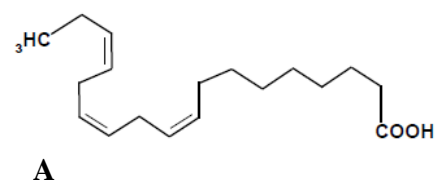


Figure 2

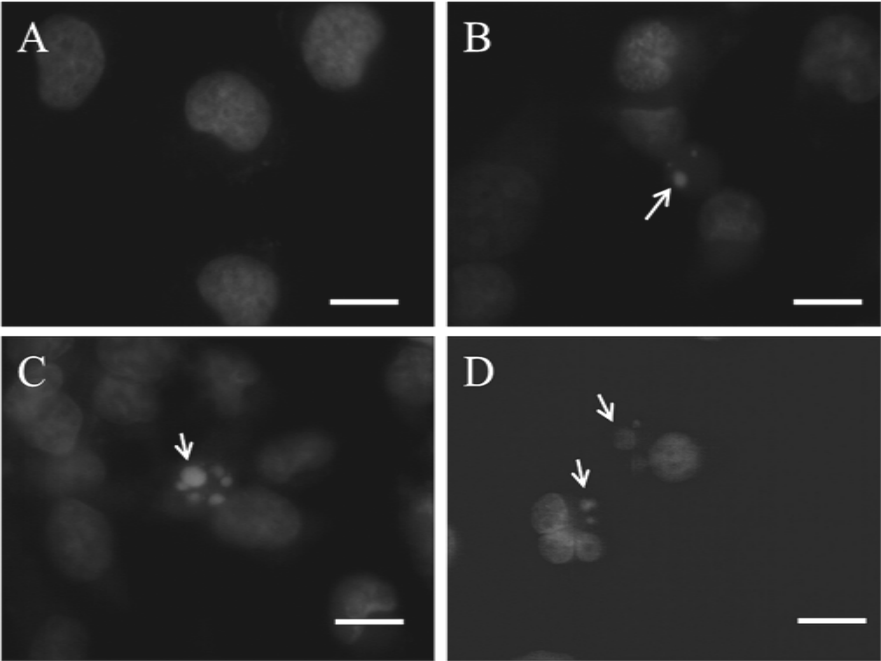


Figure 3

