REVIEW

Dietary omega-3 PUFA and health: Stearidonic acid-containing seed oils as effective and sustainable alternatives to traditional marine oils

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The daily consumption of dietary omega-3 PUFA is recommended by governmental agencies in several countries and by a number of health organizations. The molecular mechanisms by which these dietary PUFA affect health involve the enrichment of cellular membranes with long-chain 20- and 22-carbon omega-3 PUFA that impacts tissues by altering membrane protein functions, cell signaling, and gene expression profiles. These changes are recognized to have health benefits in humans, especially relating to cardiovascular outcomes. Cellular membrane enrichment and health benefits are associated with the consumption of long-chain omega-3 PUFA found in marine oils, but are not generally linked with the consumption of alpha-linolenic acid, the 18-carbon omega-3 PUFA found in plant seed oils. However, the supply of omega-3 PUFA from marine sources is limited and may not be sustainable. New plant-derived sources of omega-3 PUFA like stearidonic acid-soy oil from genetically modified soybeans and Ahiflower oil from *Buglossoides arvensis* seeds that are enriched in the 18-carbon omega-3 PUFA stearidonic acid are being developed and show promise to become effective as well as sustainable sources of omega-3 PUFA. An example of changes in tissue lipid profiles associated with the consumption of Ahiflower oil is presented in a mouse feeding study.

Keywords:
Cardiovascular disease / Fish oil / Inflammation / Stearidonic acid / Vegetable oil

1 Introduction

The increasing interest in incorporating omega-3 PUFA into the diet has been driven by the extensive literature indicating that these dietary PUFA promote health and disease prevention [1–6]. This literature includes in vitro cell-based and animal-feeding studies, as well as observational studies and randomized controlled trials in humans. Omega-3 PUFA are naturally enriched in a number of common foods such as fatty fish like salmon or tuna as well as in several common plant-derived oils like canola and soybean oils. Widely available dietary supplements such as fish oils and flax seed oil are also rich sources of omega-3 PUFA while other foods including eggs, dairy products, and baked goods that have been supplemented with omega-3 PUFA are now increasingly available [7].

The heightened desire for consumers to incorporate omega-3 PUFA into their diets and for food manufacturers to enrich their products with these fatty acids has often led to the erroneous impression that all omega-3 PUFA are equally effective in the promotion of health [8]. This desire has also led to a looming problem of the demand for these fatty acids outpacing the supply [9–11]. Therefore, there is a need for sources of omega-3 PUFA that are both renewable and that have measurable health benefits.

This review provides an overview of the different types of omega-3 PUFA, the mechanisms by which they impact

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Abbreviations: AA, arachidonic acid; **ALA**, alpha-linolenic acid; **DPA**, docosapentaenoic acid; **DHA**, docosahexaenoic acid; **EPA**, eicosapentaenoic acid; **PGE_2**, prostaglandin E_2; **SDA**, stearidonic acid

on cell and tissue function and the effects of their consumption on indices of health. Given that the supply of omega-3 PUFA from traditional marine sources may be unsustainable, a review is presented of studies investigating oils rich in stearidonic acid (SDA), a nontraditional omega-3 PUFA that may represent the next generation of dietary omega-3 PUFA. These SDA-rich oils have been developed in genetically engineered plants like canola or soybeans [12–14], but are also naturally produced in the seeds of plants from the *Boraginaceae* family [15,16]. New data are presented showing the impact of the consumption one such oil, Ahiflower™ oil from the seeds of *Buglossoides arvensis*, on the fatty acid composition of tissues in mice.

2 Omega-3 PUFA structures and metabolism

The omega-3 PUFA form a family of fatty acids that are essential nutrients since they cannot be generated by de novo biosynthesis in vertebrate animal species including humans. Structurally, omega-3 PUFA are distinguished from all other fatty acids by the presence of a double bond at the third carbon from the methyl (or omega) end of the molecule (Fig. 1). The parent or simplest omega-3 PUFA is alpha-linolenic acid (ALA). This 18-carbon fatty acid possesses three double bonds and is commonly found in plant-derived dietary oils such as flax, canola, and soybean oils. The other common dietary omega-3 PUFA are the longer chain and more unsaturated PUFA that are usually associated with fish and fish oils. These fatty acids include the 20-carbon eicosapentaenoic acid (EPA) and the 22-carbon docosapentaenoic acid (DPA) that each possesses five double bonds, and the 22-carbon docosahexaenoic acid (DHA) that possesses six double bonds.

Following their consumption, EPA, DPA, and DHA are preferably utilized for incorporation into cellular membrane phospholipids where they participate in the proper functioning of membrane lipid bilayers. Humans express the enzymes necessary for the conversion of dietary ALA to any of the other members of the omega-3 family of PUFA (Fig. 1). However, populations consuming typical Western diets do not carry out this conversion very efficiently [8, 17–19]. Consequently, following the consumption of foods or supplements containing ALA, very little if any EPA, DPA, or DHA accumulate in our cells and tissues. Since the beneficial health effects of dietary omega-3 PUFA are largely associated with the changes in tissue membrane fatty acid composition that accompany their consumption, most of the health benefits attributed to the consumption of fish oils are not attained with the consumption of vegetable oils containing ALA as the sole source of omega-3 PUFA. Although some beneficial biological activity has been associated with the consumption of ALA, any health benefits are independent of its conversion to EPA, DPA, or DHA [8, 20, 21].

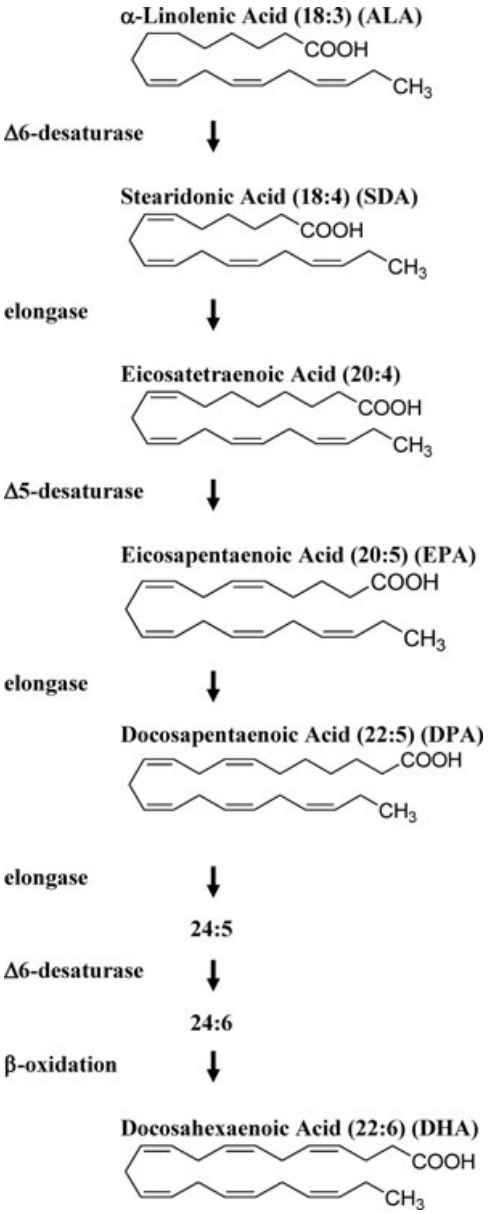

Figure 1. The metabolic pathway of omega-3 PUFA with corresponding structures. The enzymes responsible for each metabolic step (indicated by an arrow) are shown on the left side of the figure. Desaturases add a double bond at a specific site on the molecule, while elongases add two carbons to the carboxyl end of the molecule.

2.1 Incorporation into cell membranes

Dietary omega-3 PUFA are incorporated into cell membranes in all tissues of the body and changes in cell membrane fatty acid composition occur within days of daily consumption of these fatty acids [22]. Membranes from some tissues like the retina, brain, and the myocardium are very enriched in these fatty acids; e.g. omega-3 PUFA constitute approximately 30% of all fatty acids associated retinal

photoreceptor outer segment membranes [23]. That these and other cells preferentially incorporate these minor dietary fatty acids into their membranes indicates that these PUFA are important for the proper functioning of the cell. In fact, most cellular membranes accumulate omega-3 PUFA in amounts that exceed their proportional content in the diet.

Cell membranes are dynamic structures whose phospholipid composition is constantly remodeled and dietary omega-3 PUFA compete with other fatty acids for incorporation into cellular membranes. This remodeling of PUFA in cellular phospholipids, termed the Lands cycle, is characterized by the hydrolysis of saturated or monounsaturated fatty acids from phospholipids by a phospholipase A_2 followed by a reacylation with PUFA by a reaction catalyzed by a lysophospholipid acyl-CoA transferase [24, 25]. Several phospholipases A_2 and lysophospholipid acyl-CoA transferases having different characteristics and substrate specificities have been recently discovered and their differential expression is likely responsible for the diversity of the fatty acid composition in different tissues [26–28]. However, the combinations of enzyme isotypes that are responsible for different tissue fatty acid distribution patterns have not yet been determined. This cycle is supplied with several substrates obtained from the diet, and therefore the ultimate composition of fatty acids in membrane phospholipids is the net result of the specificity of the enzymes expressed in a given cell type or tissue, and the supply of substrates that, in the case of omega-3 PUFA, are ultimately determined by dietary intake.

3 Molecular mechanisms of action

3.1 Lipid mediators of inflammation

Omega-3 PUFA compete with the omega-6 family of essential PUFA for the enzymes that catalyze the remodeling of cellular phospholipids and therefore compete for incorporation into cellular phospholipids [29]. Arguably the most important of all cellular PUFA is the omega-6 family member arachidonic acid (AA). When cells are activated, AA is hydrolyzed from membrane phospholipids and is enzymatically converted into potent oxygenated signaling compounds that include prostaglandins and leukotrienes [30–32]. These compounds impact on a number of functions including the regulation of gastric secretions, bronchoconstriction, the signaling of pain, and the activation of platelets and leukocytes, among others. Notably, leukocyte activation is fundamental to inflammation, a process that is vital to immunity, but which is also intimately involved in chronic inflammatory diseases like asthma, rheumatoid arthritis, inflammatory bowel disease, and cardiovascular diseases, to name a few. In fact, the biosynthesis and action of AA-derived compounds have been targeted by the pharmaceutical industry because of their important role in the progression of inflammatory diseases [31–34]. Dietary omega-3 PUFA directly impact on the metabolism of AA-derived compounds because they compete with AA for the enzymes responsible for its incorporation into membrane phospholipids as well as those that catalyze the biosynthesis of prostaglandins and leukotrienes [35, 36]. Therefore, the consumption of foods enriched in omega-3 PUFA can diminish the potential for leukocytes or other cells to synthesize these potent AA-derived mediators of inflammation [29].

Perhaps more importantly, the EPA and DHA that are incorporated into cellular membranes are themselves transformed into recently identified biologically active oxygenated compounds that include the resolvins, protectins, and maresins [37, 38]. These omega-3 PUFA-derived compounds are lipid mediators that actively participate in the resolution of inflammation, a key mechanism that limits inflammation-induced tissue damage and ultimately the severity of chronic inflammatory diseases. Therefore, the consumption of foods containing omega-3 PUFA that enrich cellular membranes with EPA and DHA is believed to contribute to the dampening of chronic inflammation through the biosynthesis of these proresolving compounds [39].

3.2 Membrane proteins and gene expression

In addition to playing a role as precursors to bioactive lipid molecules, the omega-3 PUFA stored in cell membranes also affect the physicochemical properties of the membrane lipid environment, including that of lipid rafts and calveolae that assemble proteins and receptors that are critical to many cellular functions including signal transduction [40]. For example, omega-3 PUFA suppress protein assembly and signaling at the immunological synapse in T cells by altering raft formation [41], and the enrichment of membranes with omega-3 PUFA perturbs toll-like receptor 4 recruitment thus diminishing LPS-induced inflammatory responses [42]. Changes in the lipid microenvironment also impact on membrane-associated proteins like ion channels that control the voltage-gated sodium and calcium currents associated with the excitation of myocytes and contraction of the heart. Omega-3 PUFA inhibit the activity of a number of cardiac ion channel proteins [43] and this has been proposed to contribute to their anti-arrhythmic properties in animal studies and may explain the rapidity of the onset of protective effects on coronary heart disease mortality reported in clinical intervention trials [44, 45].

Omega-3 PUFA themselves are also ligands that directly bind to nuclear receptors that impact on gene expression. Indeed, they interact with the retinoid X receptor and peroxisome proliferator-activated receptor-gamma and modulate the activity of other transcription factors such as nuclear factor kappa B [46–49]. Such effects on the control of gene transcription is associated with the modulation of intracellular signaling pathways, the synthesis of cytokines that are involved in the amplification of the inflammatory response [48, 50]

and the expression of adhesion proteins that participate in leukocyte-endothelium interactions [51].

It should be stressed that the effects of omega-3 PUFA should not be compared to pharmacological agents that obstruct a specific target, but rather viewed as a shift in functionality, signaling, or gene expression to a phenotype of reduced reactivity in response to external stimuli. Altogether, the general shift to a condition of lessened responsiveness and reactivity in cells and tissues enriched with omega-3 PUFA likely explain the health-promoting properties of these dietary oils.

4 Clinical studies

The initial clinical observation associating health benefits with the consumption of omega-3 PUFA was reported over 40 years ago in populations of western Greenland whose dietary intake of large quantities of marine PUFA was associated with very low levels of plasma triglycerides and a low incidence of ischemic heart disease [52, 53]. Since then, a large literature investigating the impact of dietary omega-3 PUFA on human health and disease prevention has been generated. Given the action of omega-3 PUFA on cellular mechanisms associated with inflammation and cell signaling, clinical investigations have largely concentrated on chronic inflammatory diseases including cardiovascular diseases [1–5, 38, 54], although strong evidence has emerged for a role of DHA in retina and brain development and function [47, 55, 56].

The strongest evidence for beneficial health effects of omega-3 PUFA is related to cardiovascular outcomes [2, 44, 54, 57–60]. Metaanalyses of the numerous randomized controlled trials and prospective observational studies investigating fish or long-chain omega-3 PUFA consumption and risk of cardiovascular disease outcomes indicate that consumption significantly reduces coronary heart disease mortality, including sudden cardiac death and fatal myocardial infarction [2, 61]. Importantly, cardiovascular outcomes in cohorts from large prospective studies have been associated with tissue long-chain PUFA content [58, 60, 62], an objective measure that is more reliable than diet questionnaires that are often utilized to assess dietary intake. These studies have shown that plasma long-chain omega-3 PUFA content has a strong inverse correlation with risk of sudden death in individuals without a history of cardiovascular disease, and with risk of congestive heart failure. These correlations are particularly striking given that individuals in the lowest quartile for plasma omega-3 PUFA concentrations show approximately two to three times the risk compared to individuals in the highest quartile.

Although the relationship between long-chain omega-3 PUFA and cardiovascular outcomes is the most widely accepted, several controlled randomized trials investigating dietary supplementation with long-chain omega-3 PUFA have also shown positive outcomes in joint inflammation. More specifically, metaanalyses and systematic reviews of published trials in patients with rheumatoid arthritis showed significantly decreased joint pain, morning stiffness, and anti-inflammatory drug use in patients supplementing their diets with long-chain omega-3 PUFA [6, 63, 64]. Unlike the risk of coronary heart disease death that appears to be reduced with modest consumption (250–500 mg/day) of long-chain omega-3 PUFA, amelioration of arthritic symptoms are typically achieved with higher intakes of at least 2.7 g of EPA + DHA per day.

Finally, experimental models show that long-chain omega-3 PUFA impact physiological processes such as inflammation, cell proliferation, angiogenesis, and signaling mechanisms that influence the immune system and the pathogenesis of cancer. Accordingly, omega-3 PUFA have shown promising results on carcinogenesis in vitro and in animal studies [65–69]. Although some epidemiological evidence suggests that the consumption of omega-3 PUFA may reduce cancer risk in humans, there is currently little overall evidence to support that the consumption of dietary omega-3 PUFA decreases cancer incidence in humans [70, 71]. However, whether these nutrients alter the progression of cancer or are efficacious adjuvants to cancer therapy in humans has not yet been adequately evaluated and remains to be determined [71].

5 Sources of dietary omega-3 PUFA

The now well-documented effects of dietary omega-3 PUFA on human health have led government agencies in many industrialised countries as well as organizations like the American Heart Association or the World Health Organization to recommend the regular consumption of foods containing EPA + DHA [7, 61, 72]. However, the types of omega-3 PUFA found in fish are minor dietary constituents in populations consuming Western diets [7, 73] and are consumed in amounts that are inferior to recommended intakes. Despite these lower than optimal intakes, the demand for these fatty acids has continually increased over the last decade. Increasingly, food manufacturers are fortifying their products with omega-3 PUFA, mostly in the form of EPA and DHA from fish oils [7]. However, this increased demand for omega-3 PUFA has occurred while global fish oil supplies have diminished and projections indicate that demand will soon outpace supply [9–11]. Alternative sources of omega-3 PUFA have been sought after to meet this demand, not only for human consumption but also as a feed ingredient in aquaculture that commands a significant share of the global fish oil production.

A number of alternative sources of omega-PUFA have been or are being developed. Krill oil, a phospholipid-based oil that is rich in EPA and DHA, is increasingly available; however, as with other marine oils that are derived from commercial fisheries, the sustainability of krill oil production is uncertain [74]. Microorganisms like the algae *Crypthecodinium cohnii* produce oils that are enriched in DHA [75]. Although these represent an environmentally sustainable source of oil,

omega-3 PUFA derived from such cell cultures are expensive to produce and are thus unlikely to penetrate food markets to the extent that is required to attain recommended intakes on a population basis.

Perhaps the most obvious alternative to fish oils that can be produced in an economical and sustainable fashion is plant-derived oils. A number of plants produce seed oils that are enriched with ALA such as Flax (approximately 50% ALA), soy (7% ALA), and canola (9% ALA). However, as indicated earlier, the consumption of ALA has little or no measurable impact on tissue EPA, DPA, or DHA levels and the associated health benefits are not well established [8, 20, 21]. A number of transgenic varieties of common plants such canola, soybean, and safflower have been developed that produce seed oils that are enriched with EPA or DHA [76–78]. Although these plants represent an effective and potentially sustainable source of omega-3 PUFA, many of these oils are years away from commercial production and the negative perception of transgenic products in certain geographical areas may limit their uptake.

A novel plant-derived omega-3 PUFA that has generated recent interest is SDA [79]. This 18-carbon omega-3 PUFA is the immediate product of the conversion of ALA catalyzed by the delta-6 desaturase (Fig. 1). Importantly, this metabolic step is not effective in humans and is the reason why ALA is poorly converted to longer chain more unsaturated omega-3 PUFA following its consumption [8, 17–19]. Because of this bypass of a metabolic block in the pathway, the consumption of oils rich in SDA can result in an enrichment of tissues with longer chain PUFA like EPA, DPA, or DHA.

SDA is a minor constituent of many fish oils but is also found in the seeds of a number of plants, especially species from the Boraginaceae family [15, 16]. Few seed oils containing SDA have been produced for human consumption; blackcurrant seed oil (2–4% SDA) and *Echium plantagineum* seed oil (echium oil, 12–14% SDA) are currently produced commercially. A new plant oil extracted from the seeds of *B. arvensis* is a rich source of SDA (20% SDA) and is being developed for commercial production under the trade name Ahiflower™ oil. Finally, canola and soybeans [13,80] have also been genetically modified to produce seeds enriched in SDA (SDA-soybean, 20–30% SDA) [81], however, similar market barriers may also exist for these genetically modified crops. A comparison of the fatty acid profiles of these SDA-containing oils is shown in Table 1.

Table 1. Typical fatty acid composition of SDA-containing oils

Fatty acid	Percent of total fatty acids			
	Black currant[a)]	Echium[b)]	SDA-Soy[c)]	Ahiflower
16:0	9.3	7.1	12.9	4.5
18:0	2.1	3.7	4.5	1.8
18:1 (*n*-9)	16.6	15.4	20.1	8.5
18:2 (*n*-6)	41.9	18.8	24.4	12.5
18:3 (*n*-6)	13.8	11.0	6.1	6.0
18:3 (*n*-3)	12.9	28.4	9.9	41.5
18:4 (*n*-3)	2.6	12.5	19.9	20.0

Only major fatty acids are shown.
a) Ref. [82].
b) Ref. [100].
c) Ref. [81].

5.1 Dietary stearidonic acid-containing oils impact tissue PUFA content in animal studies

A number of animal studies have investigated the impact of dietary SDA on tissue PUFA content. Aside from an early study in rats using black currant seed oil [82], rodent studies have used either echium oil [83,84] or SDA ethyl esters [85–89] as a dietary source of SDA. These rodent studies have clearly shown significant increases in EPA and DPA content in plasma, erythrocytes, liver, heart, or intestines. The extent of the increase varies depending on the intake of SDA and the fatty acid composition of the control diet to which the comparison is made. In some but not all reports, the consumption of SDA also resulted in slight elevations in tissue DHA content compared to controls.

While animal studies with dietary fatty acids can be predictive of the impact that may be observed in human trials [90], unfortunately the fatty acid composition of the background or control diets in animal studies that contain palm [83], safflower [86, 87] or soybean oil [91] as the sole source of dietary lipids are not relevant to typical human diets [73, 92] making direct comparisons or translations of the extent of tissue enrichment to humans difficult. Similarly, the replacement of most if not all of the background dietary lipid solely with the test oil [83, 86, 87, 91, 93] is not feasible in the human context, again limiting the ability to translate results to humans. The use of these extremes in dietary lipid intakes also renders comparisons between individual animal studies very difficult.

A very controlled study in the $Apc^{Min/+}$ mouse model allowed direct comparisons of the impact of dietary SDA- and EPA-ethyl esters on intestine fatty acid profiles [88]. Dietary SDA-ethyl esters significantly increased intestinal EPA and DPA content. As may be expected, SDA was less effective than dietary EPA-ethyl esters but still attained tissue EPA and DPA content that were 36 and 53%, respectively, of those achieved with an equivalent intake of EPA-ethyl esters. The dietary SDA-ethyl ester had no effect on intestinal DHA content. In both neonatal [84] and adult [83] rat models consumption of dietary echium oil enriched tissues with EPA, DPA, and DHA, but to a lesser extent than that measured following consumption of similar quantities of fish oils (containing both EPA and DHA) that were between 1.2- and fivefold more effective depending on the tissue measured.

Comparable results were observed in nonrodent mammals where consumption of echium oil enhanced longer chain omega-3 PUFA content in the meat of chickens by two to threefold compared to rapeseed oil-fed controls [93], while dietary SDA-soy oil enriched the pool of EPA, DPA, and DHA

Table 2. Impact of the consumption of SDA-containing oils on tissue long chain omega-3 PUFA content in humans

Reference	Intake of SDA; duration of supplementation	Source	Subjects consuming SDA-containing diets	Cell type or tissue	Fold change from baseline		
					EPA	DPA	DHA
James et al. [18]	0.75 g/day; 3 weeks	SDA-EE	$N = 15$, Healthy adults	Erythrocytes	1.21[b)]	1.03	0.96
	1.5 g/day; 3 weeks			Plasma	1.42[b)]	1.16[b)]	0.96
			$N = 15$, Healthy adults	Erythrocytes	1.5[b)]	1.09	0.93
				Plasma	1.87[b)]	1.27[b)]	0.96
Surette et al. [100]	1.87 g/day; 4 weeks	Echium oil	$N = 11$, Hyper-triglyceridemic adults	Plasma	2.9[b)]	1.73[b)]	1.01
				Neutrophils	5.0[b)]	2.0[b)]	1.29
Miles et al. [98, 99][a)]	1.0 g/day; 12 weeks	Echium oil	$N = 8$–12, healthy young men	Plasma	1.33	nd	1.12
				PBMC	2.25[b)]	nd	1.61[b)]
Harris et al. [95]	3.66 g/day; 16 weeks	SDA-soy	$N = 11$, Overweight healthy adults	Erythrocytes	2.88[b)]	nd	1.0
Lemke et al. [97]	4.2 g/day; 12 weeks	SDA-soy	$N = 54$, Overweight healthy adults	Erythrocytes	2.23[c)]	1.34[c)]	0.94
Krul et al. [96]	5.2 g/day; 12 weeks	SDA-EE	$N = 17$, healthy adults	Erythrocytes	2.71[c)]	1.47[c)]	1.03
	2.6 g/day		$N = 14$		2.18[c)]	1.40[c)]	1.0
	1.3 g/day		$N = 16$		1.89[c)]	1.33[c)]	1.02
	0.43 g/day		$N = 18$		1.11	1.08	0.94

SDA-EE = SDA ethyl esters; PBMC = peripheral blood mononuclear cells; nd = not determined.
a) Same cohort reported in both publications.
b) Significantly different from baseline.
c) Significantly different from control diet.

in edible meat tissues by 6.3, 4.8, and 2.5-fold, respectively, compared to soybean oil-fed controls [91]. In dogs consuming SDA-ethyl esters in an amount that was described as comparable to a human intake of 13.5 g of SDA per day, erythrocyte and heart EPA content significantly increased 3.6- and 11.5-fold, respectively, compared to the presupplementation baseline values. Surprisingly, there were no differences in tissue DPA content, while heart DHA increased 1.7-fold [94].

Altogether, despite the difficulties associated with different dosing regimens and control diets as well as the use of different animal models and measurements performed in different target tissues, animal studies generally indicate that dietary SDA effectively enriches tissues in EPA and DPA, and to some extent in DHA.

5.2 Human trials with dietary stearidonic acid-containing oils

A number of human clinical trials have also been conducted investigating the impact of dietary oils containing SDA on tissue fatty acid composition [18, 95–100]. Since no long-term interventional or observational studies of SDA consumption have been conducted, tissue EPA, DPA, and DHA content can serve as a surrogate marker of potential health benefits given the relationship between tissue long-chain omega-3 PUFA content and indices of cardiovascular health and morbidity.

These human studies investigating the consumption of SDA-ethyl ester, echium oil or SDA-soy oil have all shown that tissue EPA content is significantly elevated following the consumption of SDA-containing oils (Table 2). Earlier studies reporting consumption of black currant oil are not included in the Table since this oil is not a rich source of SDA [101]. Most studies reported erythrocyte EPA and DHA content that can be utilized to calculate the omega-3 index, a proposed risk factor for coronary heart disease [102]. Using this measure, the consumption of SDA-containing oils would be predicted to be cardioprotective. EPA content was also significantly elevated in plasma, peripheral blood mononuclear cells, and peripheral blood polymorphonuclear cells in subjects consuming echium oil or SDA-ethyl esters [18, 98–100]. As with animal studies, dietary SDA had little impact on blood DHA levels; however, in those studies where total tissue fatty acids were reported, DPA levels were also significantly elevated in subjects consuming SDA-containing oils.

In humans, preformed dietary EPA-ethyl esters are approximately 3-times more efficient than dietary SDA-ethyl esters at enriching erythrocyte and plasma phospholipids with EPA [18, 96]; however, the efficiency of conversion to EPA appears to vary depending on the daily intake of SDA. In other studies comparing SDA-soy with EPA-ethyl esters, similar results were obtained where preformed EPA is more effective at enriching tissues with long-chain omega-3 PUFA [95, 97]. As expected, consumption of ALA-ethyl esters in these studies

had no significant impact on long-chain omega-3 PUFA [18]. Interestingly, dietary echium oil effectively decreased serum triglyceride concentrations in hypertriglyceridemic patients in a small open-labeled trial [100], suggesting that SDA-containing oils may share the established hypertriglyceridemic effects of dietary fish oils [3]. This observation requires confirmation in blinded placebo-controlled trials.

Altogether, human studies investigating the impact of dietary SDA-containing oils indicate that their consumption is associated with an increase in the EPA and DPA content of plasma and various blood cells. The question that remains is whether dietary SDA has beneficial health effects. In the absence of interventional or observational studies, prospective studies showing that the incidence of congestive heart failure [60] and of sudden death from cardiac causes [58] are significantly reduced when plasma EPA or plasma 20- and 22-carbon omega-3 PUFA content are increased by as little as 1.5-fold suggest that the inclusion of practical quantities of SDA-containing oils in the diet may reduce the risk of adverse cardiovascular events.

6 Investigation of dietary Ahiflower™ oil on tissue fatty acid profiles in mice

Rodent studies investigating dietary SDA have utilized SDA-ethyl esters, echium oil, or SDA-Soy oil as a source of SDA. Unfortunately, several of these studies utilized control or background diets highly enriched in linoleic acid (18:2 omega-6) or devoid of omega-3 PUFA like ALA, resulting in a fatty acid composition and lipid content that does not resemble that of typical human diets. The interspecies comparisons of dietary PUFA are more reliable when rodents are provided human equivalent doses of fatty acids based on energy consumption [90]. We therefore undertook a small feeding study in mice to evaluate effect of the consumption of Ahiflower™ oil on tissue fatty acid profiles in liver, brain and intestines using diets that were allometrically scaled to resemble human equivalent diets.

Ahiflower™ oil was utilized as a source of dietary SDA since this oil possesses the highest content of SDA other than oils extracted from transgenic plants (Table 1), and has not been previously evaluated in animals. Since Ahiflower™ oil is also rich in ALA, a control diet enriched in ALA from flax seed oil was utilized in order to isolate the impact of dietary SDA on a background diet that contains ALA.

6.1 Methods

BALB/c mice (19–21g) were separated into two groups of five animals. The Control group consumed a modification of the Monsanto US17 Rodent Diet supplemented with 0.1 g arachidonate ethyl ester/Kg of diet (Research Diets) that was designed to closely represent the typical North American diet with respect to energy distribution and fatty acid content (Table 3) [88]. The Ahiflower™ group consumed a diet containing Ahiflower™ oil providing 1% of energy supplied as SDA. The total energy from fat was equivalent in both groups with the SDA largely replacing oleic acid in the control diet. The study was conducted at the Université de Moncton animal facility that is certified by the Canadian Council on Animal Care. Protocols were approved by the institution's Animal ethics review committee under protocol 11–07.

Table 3. Energy distribution and fatty acid composition of the control diet and of the Ahiflower diet consumed by mice for 4 weeks

Component	Control diet	Ahiflower diet
	Percent kilocalories	
Protein	16.0	16.0
Carbohydrate	50.3	50.3
Fat	33.7	33.7
	Fatty acids (% total)	
C14:0	0.6	0.5
C16:0	24.5	24.6
C18:0	11.7	11.6
C18:1 *n*-9	39.6	35.4
C18:1 *n*-7	0.9	0.8
C18:2	17.3	16.3
C18:3 *n*-6	0.0	0.6
C18:3 *n*-3	5.1	6.9
C18:4 *n*-3	0.0	3.0
C20:4 *n*-6	0.1	0.1

The animals consumed diets ad libitum for 4 weeks. Body weights were recorded weekly. Mice were then sacrificed and the liver, brain, and small intestine were collected, snap frozen in liquid nitrogen, and stored at –80°C. Tissues were thawed and immediately homogenized in ice-cold saline. For small intestines, the homogenization buffer also contained 1 mM indomethacin to prevent the production of prostaglandins during sample preparation. Lipids were extracted from homogenates and FAMEs were prepared and measured by GC with flame ionization detection [22]. Endogenous prostaglandin E_2 (PGE_2) content in the 10 000 × *g* supernatant of small intestine homogenates was measured using Correlate-EIA kits (Assay Designs, Ann Arbor, MI).

6.2 Results and discussion

During the 4-week feeding period, there were no differences in animal weights or general appearance between the two experimental groups. Tissue EPA and DPA content were significantly increased in both the liver and the intestine of animals consuming the Ahiflower™ diet (Table 4). The increases in intestinal EPA and DPA were smaller than those previously reported by Petrik et al. [88] who supplemented mouse diets with approximately three times more SDA in the form of SDA-ethyl esters; therefore the present results, including a lack of effect on intestinal DHA content, are comparable to those previously reported. Similarly, an increase in liver EPA,

Table 4. Fatty acid composition of liver, intestine, and brain in animals consuming the control diets and the Ahiflower oil diets. Animals consumed the diets for a total of 28 days

	Liver		Intestine		Brain	
Fatty acid	Control	Ahiflower	Control	Ahiflower	Control	Ahiflower
			(Percent of total)			
16:0	25.0 ± 0.4	26.1 ± 0.2	16.6 ± 1.3	15.8 ± 0.4	22.3 ± 1.5	22.0 ± 2.0
18:0	10.7 ± 0.5	12.3 ± 0.6	24.1 ± 0.9	27.0 ± 0.1	19.7 ± 0.4	19.7 ± 0.7
18:1 *n*-9	30.7 ± 0.7	24.5 ± 1.5[a)]	15.3 ± 0.8	12.8 ± 0.5	16.6 ± 2.1	17.2 ± 2.6
18:1 *n*-7	1.3 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	1.4 ± 0.04	3.3 ± 0.2	3.4 ± 0.4
18:2	14.2 ± 0.1	13.6 ± 0.3	16.2 ± 0.2	15.8 ± 0.1	0.6 ± 0.04	0.6 ± 0.1
18:3 *n*-3	0.9 ± 0.1	1.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.05	ND	ND
18:4 *n*-3	0.05 ± 0.01	0.2 ± 0.02[a)]	ND	ND	ND	ND
20:3 *n*-6	0.9 ± 0.04	1.4 ± 0.1[a)]	2.6 ± 0.2	3.2 ± 0.1[a)]	0.5 ± 0.1	0.6 ± 0.1
20:4 *n*-6	9.0 ± 0.6	9.7 ± 0.8	15.3 ± 0.6	14.3 ± 0.4	9.7 ± 0.8	9.0 ± 1.2
20:5 *n*-3	0.4 ± 0.02	1.7 ± 0.1[a)]	1.0 ± 0.1	2.2 ± 0.1[a)]	ND	0.07 ± 0.01
22:4 *n*-6	0.21 ± 0.01	0.16 ± 0.01[a)]	1.3 ± 0.1	1.1 ± 0.03[a)]	3.2 ± 0.3	2.9 ± 0.2
22:5 *n*-3	0.2 ± 0.01	0.5 ± 0.03[a)]	0.4 ± 0.05	0.8 ± 0.02[a)]	0.5 ± 0.1	0.6 ± 0.1
22:6 *n*-3	4.4 ± 0.2	5.8 ± 0.2[a)]	4.2 ± 0.3	4.5 ± 0.1	18.8 ± 1.8	18.9 ± 1.7

Values represent means ± SEM. $n = 5$ per group. ND: Mean value less than 0.05% of total fatty acids.
a) Significantly different from respective control, two-tailed *t*-test, $p < 0.05$.

DPA, and DHA is consistent with previous reports in rats consuming echium oil [83, 84]. It is difficult to make direct comparisons regarding the magnitude of the changes with these previous studies because of the significant differences in control diets and daily SDA intakes and one study was done in neonatal rats. However, the current study confirms that the liver appears to differ from other tissues since all three SDA-oil feeding studies report significant increases in DHA content. These increases in long-chain omega-3 PUFA content were observed despite the high ALA content of the control diet indicating that the SDA component in the Ahiflower™ oil was very effective at modifying tissue EPA, DPA, and DHA. Finally, during this 4-week study, no significant effect on brain PUFA composition was measured. The only other study measuring brain fatty acids in SDA-fed animals was in echium oil-fed 6-day old neonatal rats where no change in brain EPA was observed, but where small increases in DPA and DHA were measured compared to animals consuming a rat milk substitute [84].

In both liver and intestine, the content of dihomo-gammalinolenic acid (20:3 *n*-6) increased, likely due to the presence of gamma linolenic acid (18:3 *n*-6) in the Ahiflower™ oil. Dihomo-gammalinolenic acid is generally considered an anti-inflammatory PUFA since it is the precursor of the 15-lipoxygenase products 15-HETrE that exhibits anti-inflammatory properties [103]. Tissue AA was not different in the two groups of animals indicating that it was not significantly displaced by omega-3 PUFA, however, the long-chain *n*-6 fatty acid 22:4 *n*-6, which is itself a potential source of inflammatory mediators, was decreased in tissues from animals fed the Ahiflower oil diets. Despite no effect on tissue AA content, animals consuming Ahiflower™ oil produced significantly less intestinal PGE_2 (15.7 ± 1.6 versus 7.0 ± 1.2 pg PGE_2/mg protein in Control and Ahiflower™ groups, respectively; means ± SEM, $p < 0.05$ two-sided Students *t*-test) indicating that the endogenous production of cyclooxygenase-derived AA metabolites was significantly reduced.

Altogether, lipid patterns in tissues obtained from animals consuming Ahiflower™ oil are consistent with an anti-inflammatory profile. Importantly, these results were obtained with inclusion of as little as 1% SDA as total dietary energy. Based on the percentage of energy in a 2000-calorie human diet, this would be equivalent to the consumption of 2.2 g of SDA or 11 g of Ahiflower™ oil per day in a human equivalent diet, an intake that is attainable for a vegetable oil incorporated into foods.

7 Summary

In conclusion, there is abundant support for the inclusion of omega-3 PUFA in the diet as a means of promoting health and preventing disease. Evidence for this is especially strong for the association between dietary omega-3 PUFA from marine oils that enrich tissues with EPA, DPA, and DHA, and the reduced risk of adverse cardiovascular events. New plant-derived, SDA-containing oils like echium oil, Ahiflower™ oil, and oils from genetically modified plants represent new dietary sources of omega-3 PUFA that are effective at enriching tissues in long-chain omega-3 PUFA and may represent viable and effective alternatives to marine oils whose supply may not be sustainable. Importantly, the inclusion of these SDA-containing vegetable oils in the food supply may represent a means by which the intake of omega-3 PUFA can be enhanced to positively impact population health.

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